Understanding Silver Based Antimicrobials:
Mode of Action, Testing Methods, Environmental Fate, and Performance Expectations

INTRODUCTION

Silver is a heavy metal with relatively high toxicity to prokaryotes when delivered in the proper chemical form and concentration. Metallic and ionic silver (Ag^{+}) from various silver salts have been used historically in medicine primarily as topical treatments for minor skin complaints but have recently been employed in textiles and hard surfaces with claims of controlling the growth of odor causing and health significant bacteria. Silver, in its ionic form, is part of a class of antimicrobial agents know as “Leaching Biocides”. In its metallic form it is biologically inert but accumulates in both humans and the environment. For these types of agents to work, they must be transported to and diffuse into/onto the target cell. The implications of this to the environment, to higher organisms, and to adaptation of exposed microorganisms present unnecessary risks and need to be carefully examined.

Antimicrobial agents are applied onto a wide variety of fabrics and finished textiles goods to protect them from the negative effects of microbial contamination. These negative effects include stains, odors, deterioration, health, and risks associated with transfer of microbes. The choice of antimicrobial agents requires an understanding of their mode of antimicrobial action and their safety to humans and the environment.

This paper summarizes the critical issues surrounding the use of the heavy metal silver in textiles, the concerns of “nano-silver”, and compares laboratory test data between commercially produced textile products containing either leaching silver based agents (Ultra-Fresh, Silpure) or the non-leaching silane-quat based agents (EcoFresh, AEGIS).

Mode of Action and Mechanism of Resistance for Silver Ion Producing Toxicants

In bacteria, silver ions are known to react with and denature the structure of various intercellular and extracellular proteins structures. Silver ions act as antimicrobial agents by strongly binding to critical biological molecules (proteins, DNA, RNA) and disrupting their function(s). Silver is known to inhibit various enzymes critical in the respiratory chain, interfere with DNA replication, and cause metabolite efflux. The DNA chelating function critically disrupts the internal cell life processes. Metallic silver has no antimicrobial properties. It is only until the metallic silver ionizes in the presence of water that it reaches the level 1 (Ag[1]) oxidation state that it is sufficiently stable to be a relevant biocide. This biologically active state is extremely reactive and binds to proteins and bacteria alike (live or dead). Once binding to the bacteria, the silver acts as indicated above. However, the silver ions are non-specific. They will bind not only to live bacteria but also dead ones, cytoplasmic material, serum, blood, sweat, detergents, etc. This binding will no only result in the loss of antimicrobial activity but will still result in the uncontrolled release of silver into the environment. Demonstrations of this inactivation are presented below and summaries of the environmental fate and concerns follow this article. Like all leaching biocides, there is not a localized infinite supply. The silver will be released whether it finds a microbiological relevant target or not. Indiscriminate release of metallic silver into the environment is both wasteful and environmentally insensitive.

In recent years, producers of silver based agents have been able to slow the release of the ionic silver into the environment by binding the silver into the textile matrix or into zeolite carriers. While this ability to slow the release can provide for extended protection (slow release from the reservoir), this does not alter the fact that the biocide is released into the environment. Low levels of biocide release in every day use has been shown to cause bacterial resistance with other biocides. Bacterial resistance to silver is well documented in the scientific literature. The ability to adapt is transferred from bacteria to bacteria via a DNA plasmid. This adaptation mechanism not only includes an inactivating protein but also turns on two active efflux pumps which rid the cells of silver. While silver does have many sites at which it acts in the bacteria with antimicrobial action, the bacteria only need one mechanism to become resistant. The original silver resistant organism was isolated from a hospital burn ward. This is certainly a potentially serious real world problem and deserving of additional research work.

With the development of “Nano-Silver” a new and untested threat emerges. Nanoscopic particles containing silver allow for the unprecedented release of silver into the environment. This heavy dose of localized ionic silver needs to be fully examined for the human toxicity threat that may exist. Furthermore, the nanoscopic size of these particles may increase the potential threat of human exposure by access through skin by crossing the skin barrier. This threat has been recognized by many University and Environmental groups who have either petitioned governments to heavily investigate the use of Nano-silver or have called for the complete ban of the indiscriminant use of such silver products.

Antibacterial Textile Test Methods and Laboratory Performance

There are many antibacterial test methods available for use on treated textiles. The appropriate test methods are determined based on the type of biocide used. For leaching biocides, the "Zone of Inhibition" (ZOI) tests are usually employed (AATCC 147, JIS 1902, SN 19592). These tests typically provide a qualitative analysis of the extent to which a biocide effectively leaches from a given substrate (textile). Some suppliers of silver containing products claim that silver is non-leaching and durable because there is an absence of a ZOI in these test methods. This absence of a ZOI is not due to the non-leaching activity of the silver ion, but to the inability of a functional silver ion to diffuse through a nutrient agar. With the understanding of the mode of action of silver, it is obvious that this agent must migrate from the textiles surface to be effective, therefore it is a leaching agent and subject to all concerns surrounding such agents. Antimicrobial activity of commercial products as measured using the AATCC 147 test method is presented in Figure 1. Commercial textile samples prepared with Silver (Silpure), Triclosan (Microban), or the non-leaching, chemically bonded AEGIS silane-quat (EcoFresh) were tested using this zone of inhibition study. In this case, only the Triclosan treated textile displayed antimicrobial activity at some extended zone around the sample.

100% Cotton Samples	**ANTIMICROBIAL ACTIVITY** **AATCC 147** ***Zone of Inhibition***
Untreated Standard	No
Silver Treated	No
Triclosan Treated	Yes
ÆGIS Treated	No

Figure 1: Leaching activity in agar/water medium. AATCC147 using *Escherichia coli*

Lack of a zone of inhibition on silver treated samples does not alone indicate the presence of non-leaching, bonded agents but the presence of such a zone is a clear indication of leaching technology. Such zones have been shown from textiles treated with both Triclosan (Microban) and the Sanitized T99-19 products which clearly indicate that an active biocidal product is leaching from these surfaces and providing some external effect. It is unexpected to see such zones appear from articles treated with the Sanitized agent as it is claimed to be solely a non-leaching agent, however, simple verification tests prove otherwise.

Some silver biocide suppliers have recommended the use of an alternate textile test method originally designed for quantitative measurement of antimicrobial activity from non-leaching materials. This method (ASTM E2149-01) measures the antibacterial activity of treated textiles after 1 hour of contact. Due to the time needed for the leaching biocides to be released from the textile, migrate into and onto the target organism and poison the cells, textiles treated with leaching biocides generally cannot meet this standard. Most laboratories using this method for the analysis of silver treated textiles have either altered or modified the ASTM standard to provide 24 hours of contact as the action of ionic silver, while effective, is not immediate. However, with an increase in release rates in some silver treated textiles (nano-silver), it is now possible to observe significant kill within the 1 hour contact times with some select organisms. These results are presented in Figure 2.

100% Cotton Samples	**ANTIMICROBIAL ACTIVITY** **ASTM E2149** ***Dynamic Shake Flask***	
	1 hour contact	**24 hour contact**
Untreated Standard	0%	0%
Silver Treated	99.9%	99.9%
Triclosan Treated	30%	99.5%
ÆGIS Treated	99.9%	99.9%

Figure 2: Contact activity in dynamic buffer medium. ASTM E2149-01, 1 and 24 hr contact using *Escherichia coli*.

The ability of silver to leach from the treated textile into the surrounding environment can also be measured using the ASTM E2149 test method. In order to demonstrate the leaching activity of the silver treated fabric, commercially available silver treated fabric samples were tested using the ASTM E2149-01 antibacterial test by measuring the activity of a test solution that has been presoaked in the presence of treated fabric. If leaching antimicrobials are present, residual activity would be detected in this solution after it has been filtered of fabric (Figure 3).

100% Cotton Samples	**ANTIMICROBIAL ACTIVITY** **ASTM E2149 – Sec.12M** ***Solution Test***
Untreated Standard	0%
Silver Treated	99.6%
Triclosan Treated	99.5%
ÆGIS Treated	0%

Figure 3: Leaching activity in buffer medium. Solution activity using ASTM E2149-01, 1 contact using *Escherichia coli*.

Both the silver and the triclosan samples demonstrated residual antimicrobial activity after the fabric sample was removed from the suspension. This indicates that the active material has leached out of the treated textile and into the surrounding medium. No such activity was observed with the AEGIS treated sample which further demonstrates the durable, non-leaching behavior of the AEGIS antimicrobial agent.

It is always the attempt in laboratory testing to predict the performance of treated textiles in real life conditions. Many types of textile materials are treated with antimicrobial agents in order to prevent the growth of odor causing organisms. These fabrics, generally under-shirts, will be worn close to the skin which will come in constant contact with human proteins and sweat and a broad spectrum of microorganisms including both Gram negative and Gram positive bacteria , yeast and other fungi. As speed of kill also directly influences the odor causing bacteria, speed of antimicrobial action was measured using the ASTM E2149 test method. The EcoFresh AEGIS samples demonstrated very quick kill ability. Over 70% of the exposed bacteria (> 50,000,000 cfu) were destroyed with only 10 minutes of contact. After just 20 minutes, over 90% were killed (Figure 4).

100% Cotton Samples	**ANTIMICROBIAL ACTIVITY** **ASTM E2149** ***Dynamic Shake Flask***	
	10 minutes	**20 minutes**
Untreated Standard	0%	0%
Silver Treated	0%	<10%
ÆGIS Treated	71%	93%

Figure 4: Timed Contact kill activity in dynamic buffer medium. ASTM E2149-01, 10 and 20 minute contact using *Escherichia coli*.

Testing was performed using the standard ASTM E2149 test method comparing the antimicrobial activity of both SilPure and EcoFresh in the presence of typical human contaminates. Antimicrobial active silver ions are easily deactivated in the presence of proteins. Human proteins and human sweat are everyday occurrences in fabrics in close contact with the skin. In this experiment, antimicrobial activity was measured in the presence of Fetal Bovine Serum (simulated human proteins) and alkaline perspiration prepared as per AATCC 16-1997 (simulated human sweat). As predicted, the silver ions are quickly deactivated and little to no antimicrobial activity is measure with the SilPure product. These human agents had little to no affect on the EcoFresh treated samples (Figure 5).

100% Cotton Samples	**ANTIMICROBIAL ACTIVITY** **ASTM E2149** ***Dynamic Shake Flask***	
	Fetal Bovine Serum **(Protein)**	**Perspiration (AATCC 16-1997)** **(Simulated Human sweat)**
Untreated Standard	0%	0%
Silver Treated	0%	72%
ÆGIS Treated	96%	99.9%

Figure 5: Inactivation of biocidal activity by environmental contaminants. ASTM E2149-01, 1 hour contact using *Escherichia coli*.

CONCLUSIONS

Products containing either metallic silver or ionic silver have been around for ages. These products can, to varying degrees, be effective at controlling some bacteria. Silver, however, is a heavy metal and there are many concerns about its use and abuse in the environment. Understanding these limitations of each active ingredient under actual use conditions is crucial. Bioaccumulation in our environment and bodies could be a significant issue as the active antimicrobial agent readily leaches into the environment. New and improved

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"NanoSilver" has not only increased the biocidal activity of silver but improved the leaching ability. This new class of biocide is under-studied with regard to its environmental fate and human toxicity.

The document is not intended to be a full review on all aspects of the use of silver in textiles. It is, however, intended to provide the reader with critical facts when comparing antimicrobial technologies and to provoke the reader into investigating these issues further. The following articles are only a small portion of documents that discuss the pros and cons of the use of Silver in textiles. While comprehensive, there are many more articles which can discuss each of these issues. We recommend that the reader investigate not only these articles but others available on the Internet, in Trade Magazines, and Peer Reviewed publications.

Understanding the implications of the silver antimicrobial mode of action and the delivery of the active ingredient to the target cell are critical when making safe, sound antimicrobial choices.

Related Reference Material:

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