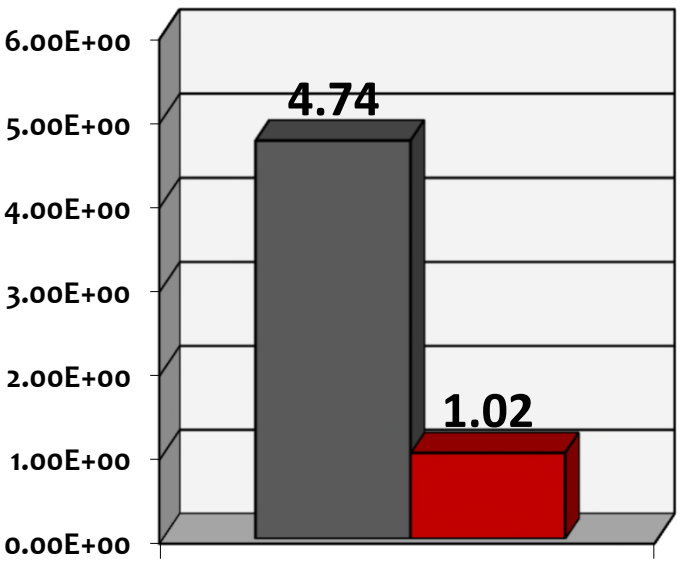
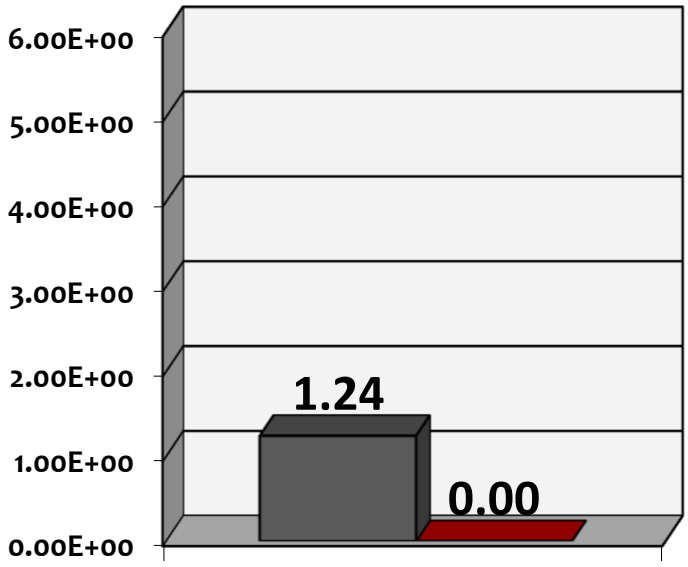
NORGANIX

Effective Against Gram-Negative Microbes

Presence of Salmonella Bacteria on the Surface of the Concrete



Presence of Salmonella Bacteria in the Interior of the Concrete



Before NORGANIX Applied

After NORGANIX Applied

Key Auburn University Laboratory Research Findings

1) Significant levels of Salmonella bacteria do in fact penetrate deep into the concrete through the capillary system!

2) NORGANIX eliminates more than 99.9% of the Salmonella bacteria both on and within the concrete.

3) Salmonella may be reintroduced to the surface of concrete where it can be treated, but the bacteria cannot reenter the concrete.

Note: This research on Gram-negative microbes (using Salmonella as the test pathogen) was published by Auburn University researchers in July, 2007. Subsequent research by Auburn has confirmed NORGANIX to be effective against Gram-positive pathogens including: Listeria. Recent testing by Auburn University has also verified NORGANIX to be effective in eliminating spore-forming microbes, including Bacillus , Clostridium and others.

What lives in your concrete?

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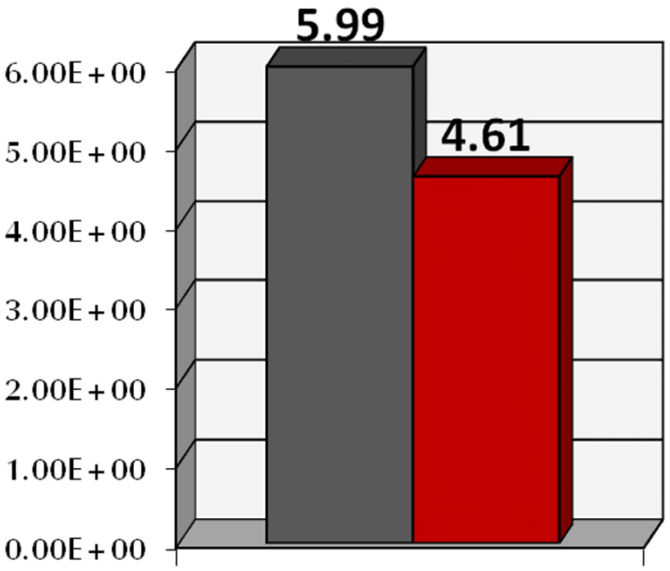
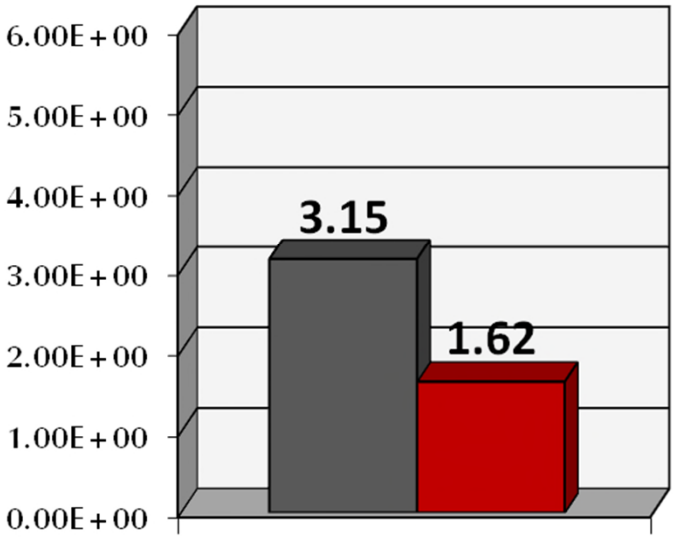
NORGANIX

Effective Against Gram-Positive Microbes

Presence of Listeria Bacteria
on the Surface of the Concrete



Presence of Listeria Bacteria
in the Interior of the Concrete



Before NORGANIX Applied

After NORGANIX Applied

Key Auburn University Laboratory Research Findings

1) Significant levels of Listeria bacteria do in fact penetrate deep into the concrete through the capillary system!

2) NORGANIX eliminates more than 95% of the Listeria bacteria on concrete and 97% within the concrete.

3) Listeria may be reintroduced to the surface of concrete where it can be treated, but the bacteria cannot reenter the concrete.

Note: This research on Gram-positive microbes (using Listeria as the test pathogen) was published by Auburn University researchers in July, 2009. Prior research by Auburn had confirmed NORGANIX to be effective against Gram-negative pathogens including: Salmonella. Recent testing by Auburn University has also verified NORGANIX to be effective in eliminating spore-forming microbes, including Bacillus , Clostridium and others.

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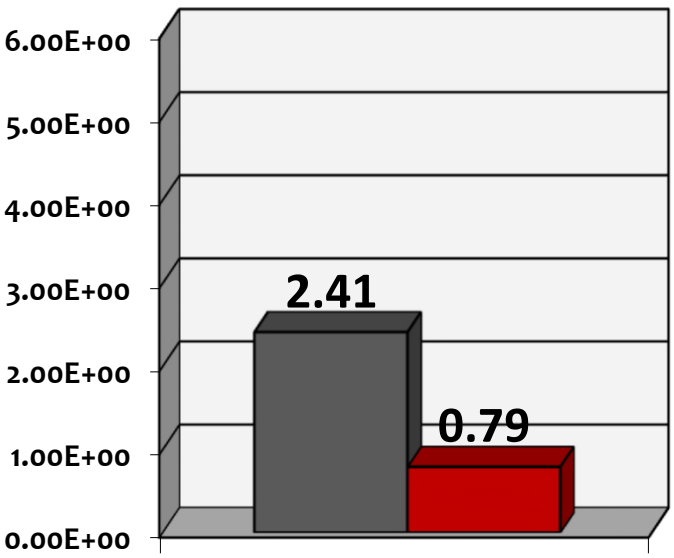
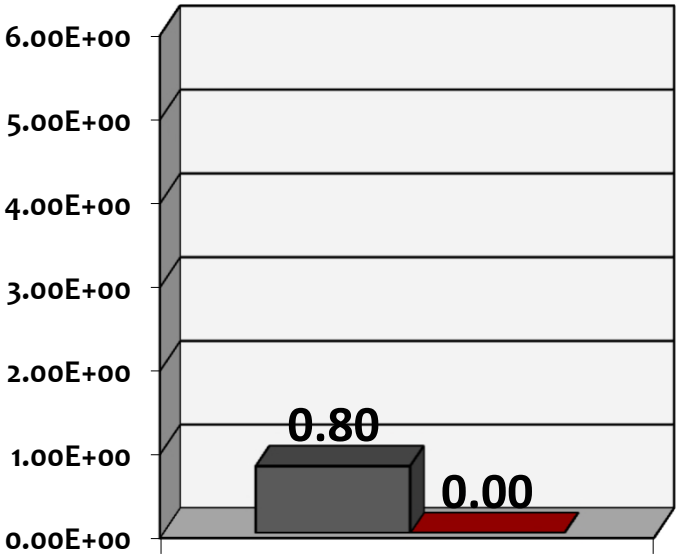
Effective Against Spore Former Microbes

Presence of Spore Formers
on the Surface of the Concrete



Presence of Spore Formers
in the Interior of the Concrete



Before NORGANIX Applied

After NORGANIX Applied

Key Auburn University Laboratory Research Findings

1) Significant levels of Spore Former microbes do in fact penetrate deep into the concrete through the capillary system!

2) NORGANIX eliminates more than 99% of the Spore Formers both on and within the concrete.

3) Spore Formers may be reintroduced to the surface of concrete where it can be treated, but the spores cannot reenter the concrete.

Note: This research on Spore Formers (using Bacillus and Clostridium as the test pathogen) was published by Auburn University researchers in March, 2010. Subsequent research by Auburn has confirmed NORGANIX to be effective against Gram-negative pathogens (Salmonella and E.coli) and Gram-positive pathogens (including Listeria).

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Auburn University Research Summary

"Preventing Salmonella Colonization in Cement Using NORGANIX"

Summary of Laboratory Research conducted by
K.S. Macklin, J.B. Hess, and D.E. Conner
Department of Poultry Science

Auburn University

July 2007

What lives in your concrete?

Abstract

Salmonella is an important food borne pathogen that is often associated with poultry. Unfortunately, the ability to properly clean and disinfect an area to remove Salmonella as well as other bacteria can be difficult. This is especially true in a constantly wet environment, such as that typically found in a poultry processing plant. In this study a commercial product (NORGANIX marketed by NORGANIX, Inc.) that claimed to kill, encapsulate or displace bacteria was tested. This product was tested on cement blocks that had been impregnated with Salmonella typhimurin.

To test the product an experiment was designed that consisted of four treatments that contained 5 blocks each. The four treatments were:

1. An unchallenged group (CON)
2. A challenged untreated group (CHAL)
3. A challenged, pre-challenged treated group (PRE), and
4. A challenged, post-challenge group (POST).

The PRE group was treated with the product according to manufacturer specifications. After 1 hour PRE, CHAL and POST were then placed in a broth that contained approximately 5 x 109 CFU/ml of a Salmonella typhimurin. After 24 hours, the blocks were removed and the POST group treated. Six hours after removal from the broth, swabs were taken of the surface from each block. After the external swabs were taken, internal swabs were performed. Swabs were done in duplicate with one swab being placed in TTB Hajna and the other being used to direct plate onto XLT4. After 24 and 48 hours of incubation, XLT4 plates were read for the presence of Salmonella. The TTB was allowed to incubate for 48 hours before being streaked onto XLT4. Salmonella CFU/cm2 counts for the directly counted XLT4 plates were transformed using log10. All of the data was analyzed using GLM with $P<0.05$ and significant means were separated using Tukey's HSD.

Salmonella was detected on the block's exterior from treatments CHAL and PRE, however there was no Salmonella with the CON and POST treatments. Salmonella was detected on the interior of the block only from the CHAL group. The results of this experiment showed that NORGANIX is an effective cement treatment that eliminates Salmonella when it is applied either before or after the cement was exposed to Salmonella.

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Introduction

Salmonella is the most significant food borne pathogen associated with poultry and its control is under increasing scrutiny by the Federal Government. Many researchers have explored options in controlling this pathogen on the farm and in the processing plant with varying degrees of success.

One area of the processing plant that has received limited attention as being a possible reservoir of this pathogen is the cement. The floor, walls and ceiling of a processing plant are made of cement that has been sealed for easy cleaning. However over time this sealant will wear off, this could allow Salmonella or other pathogens to establish themselves in the processing plant environment.

NORGANIX claims to be antibacterial, in addition it fills the capillary air passages within the cement. This is performed by a catalyst that causes the un-solidified cement to solidify, thus filling these passages. This capability should allow for better sealing of the cement that shouldn't wear off, like traditional sealants.

The purpose of this study was to determine the effect this product has on preventing Salmonella from infiltrating cement.

Materials and Methods

Cement Blocks: Cement was poured into a standard ice tray to produce 4.5 x 3.0 x 2.5 cm blocks. After solidifying, some of the blocks were treated with NORGANIX.

Salmonella: The Salmonella typhimurin that was used in this trial was initially isolated from a carcass in a poultry processing plant. For this trial the Salmonella isolate was removed from the freezer and cultured onto Blood Agar (BA). After incubation for 24-48 hours at 37C, unique colonies were then cultured onto Xylose lysine tergitol 4 agar(XLT4) for an additional 24-48 hours at 37C to verify purity. Brain Heart Infusion (BHI) Broth was inoculated and allowed to grow on a shaker incubator for 13 hours at 37C.

Inoculation of Blocks: Blocks were contaminated by placing them into the actively growing (log phase) culture of Salmonella typhimurin that was in the BHI Broth. After addition of the blocks, they were returned to the shaker incubator for an additional 24 hours.

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Materials and Methods (cont'd)

Application of NORGANIX: NORGANIX was applied either before the blocks were contaminated or immediately after removal from the Salmonella containing BHI. NORGANIX was applied according to the manufactures recommendation. Application was performed using a Wagner Paint Gun. Each surface of the block was covered with the product and allowed to dry. After 15 minutes the blocks were ready for their respective treatments.

Experimental Design: In this experiment 4 treatments were used. There were four treatments with each treatment having ten blocks. Treatment 1 had no product or bacteria exposed to the blocks. Treatment 2 had no product added, but was exposed to Salmonella as described above. Treatment 3 had the product applied to the block before exposure to Salmonella. The fourth treatment had the product applied to it after the block was exposed to Salmonella.

Salmonella Recovery: After exposure to the bacteria and in some treatments exposed to the test product, remaining Salmonella was determined. This was performed by swabbing the "top" of the cement block using two sterile swabs. One swab was placed into 2 ml of phosphate buffered saline (PBS), agitated then plated onto two XLT4 plates, while the other was placed into 10 ml of tetrathionate TT Hajna broth (TTB). After 24-48 hours at 37C for the XLT4 plates, counts were obtained. After 48 hours TTB was plated onto XLT4 and allowed to incubate as described above. After the initial swabbing of the exterior of the blocks, each block was washed in a quaternary ammonia solution. After this wash the exterior was swabbed as described above and Salmonella isolation was attempted as above. After this washing, blocks were ascetically broken open using a chisel. After each block the chisel and anvil were cleaned with 85% Ethyl Alcohol. Swabs were taken from one of the halves as previously described and Salmonella was isolated.

Count Determination: Counts obtained from the diluent that was swabbed onto XLT4 were added together then multiplied by 5. This number was then multiplied by 0.074 so that the final counts can be expressed in CFU/cm2. The counts obtained from the TTB are expressed as either positive (+) or negative (-).

Statistical Analysis: Resulting CFU/cm2 counts for the different treatments were pooled and analyzed using GLM. If significant differences were detected at a $P<0.05$ then the means were separated using Tukey's HSD test.

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Figure 1: Recovery of Salmonella from the Exterior of Cement Blocks.

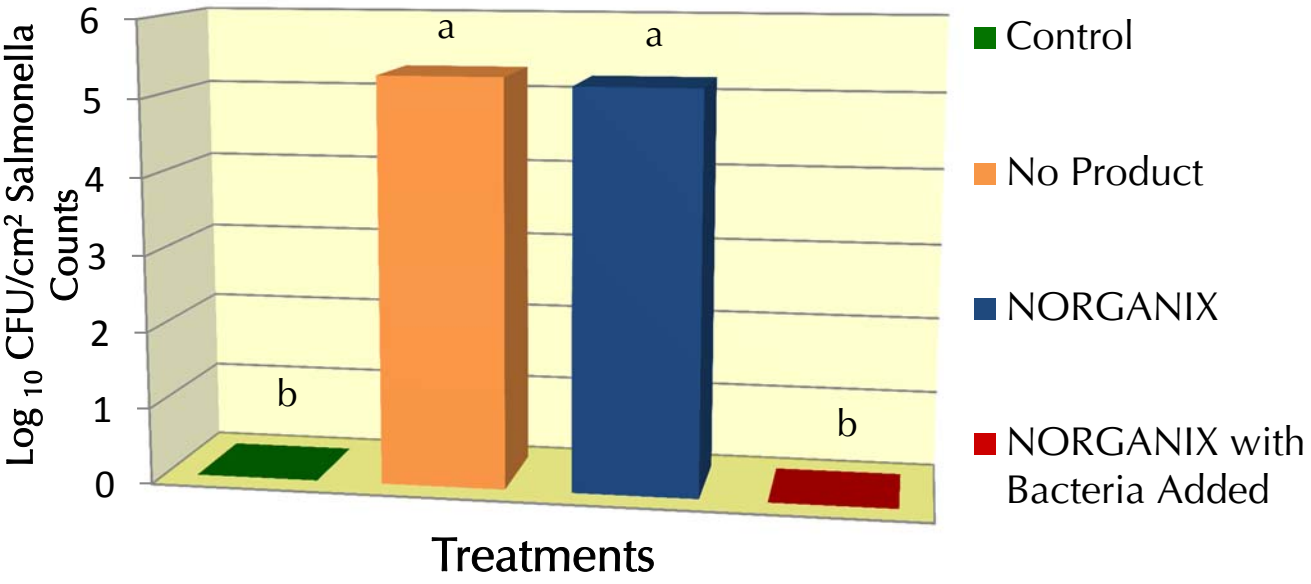
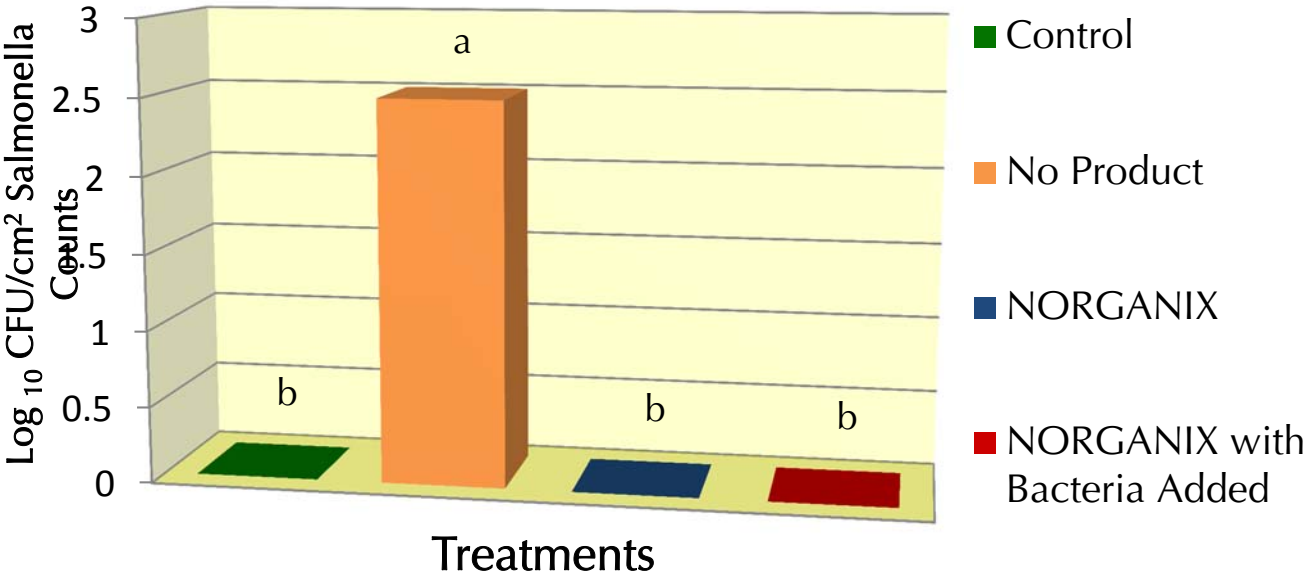


Figure 2: Average Salmonella Counts in Log_{10} CFU/cm² From the Interior of the Cement Block.



Statistical difference (P<0.05) are expressed by letter differences (a, b).

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Results and Discussion

Figure 1 shows the recovery of Salmonella from the exterior of the block. As can be observed the group that was treated with the product after exposure to Salmonella was negative. This differs significantly ($P<0.05$) from what was observed in the positive control (no product) and the group that had the product added before exposure to the bacteria. These results are not surprising since previous work by D.E. Conner (unpublished) has shown that this product has an antibacterial effect.

Washing of the blocks with quaternary ammonia removed all of the Salmonella from the surface, since none was recovered after this was performed (not shown). Interior Salmonella levels were measured in Figure 2. In the group that was treated with NORGANIX before exposure to the bacteria, it prevented colonization by sealing the block. This result is similar to what would be expected from any traditional sealant. In the group that was treated with NORGANIX after exposure, there too were no recoverable bacteria, even after enrichment. It is assumed that this product either forced the bacteria out of the cement air capillaries or the bacteria were eliminated by the products antimicrobial action.

The results from this preliminary experiment show that this product could be used in existing processing plants to eliminate bacteria that may be present within the cement's air capillary system. Further will soon be complete to confirm this product's efficacy in controlling other common food borne pathogens like Listeria, Campylobacter, E.coli, and Clostridium.

This research was first presented to the scientific community by Auburn University on July 12, 2007. This summary has been produced by NORGANIX, Inc. with the approval of the authors.

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