MOLD-CONTAMI

Mycotoxin Remov

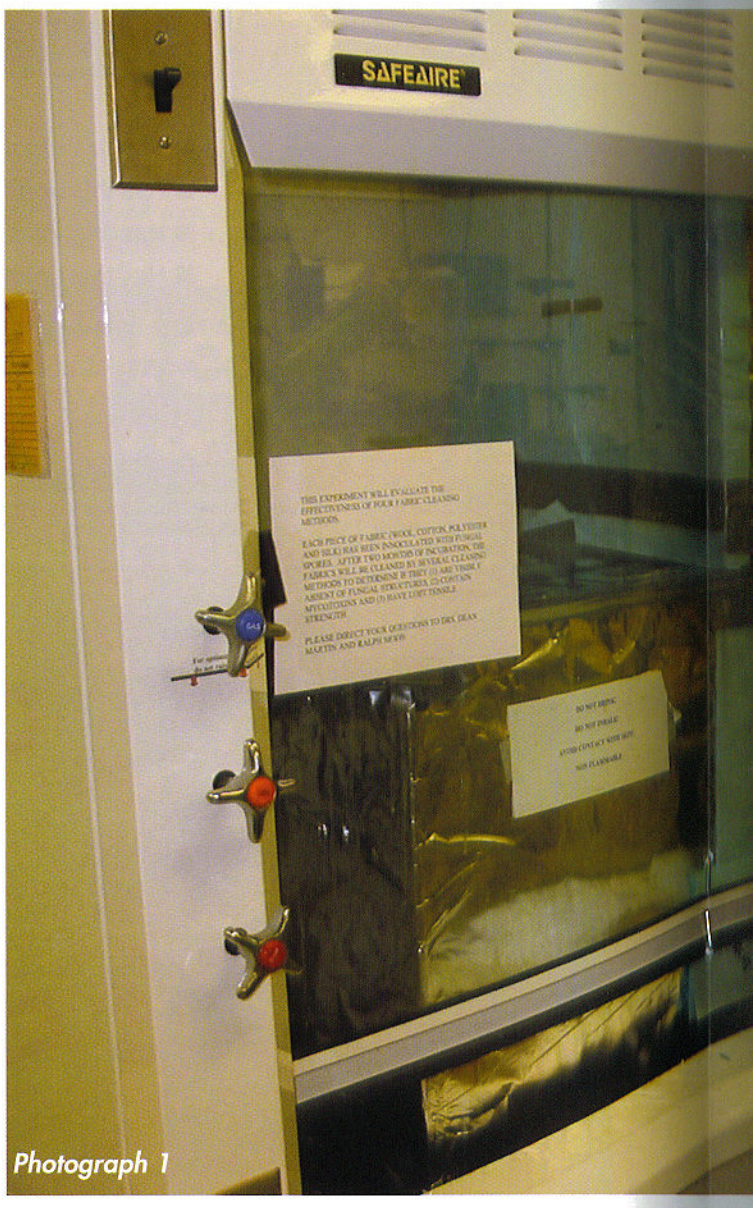
By Ralph E. Moon, Ph.D., De-Wei Li, Ph.D., and Chin Yang, Ph.D.

Editor's Note: *Part 1 of this series appeared in the October 2004 issue of Cleaning & Restoration and provided a general overview of the study and its results. Part 2 provides a more in-depth look at the mycotoxins utilized in the study, the success of their removal from the inoculated fabrics and the conclusions that can be drawn from the information gained. Both articles are based on Dr. Moon's presentations at ASCR's restoration conference, All About Contents, Damage & Solutions, in September 2004.*

Introduction

Mycotoxin exposure is a principal concern to occupants of water damaged buildings because of their association with many toxicological effects. Scientific literature links mycotoxin exposure to kidney ailments, respiratory irritation of mucosal tissues (Hintikka and Nikulin, 1998; Fung and Clark, 2004), and to a complex array of neurological impairments of short-term memory, judgment, concentration and hand/eye coordination (Rea *et al.*, 2003). Acting as immunosuppressants, mycotoxins are linked to repeated infections, especially among those in impaired buildings (Reijula and Tuomi, 2003).

Though scientists agree that comprehensive investigations of these hypotheses have not been performed, there is consensus that efforts to limit mycotoxin exposure in the air and affected contents are beneficial. Clothing that is exposed to damp environments is well suited to support microbial growth. Among the many cleaning chemicals, dilute solutions of bleach have demonstrated to be the most effective in deactivating this toxin (Wilson *et al.*, 2004). This article discusses the effectiveness of four professional cleaning methods and their ability to remove mycotoxins from four fabrics.



Photograph 1

ART 2

INATED FABRICS

oval and Transfer

Methodology

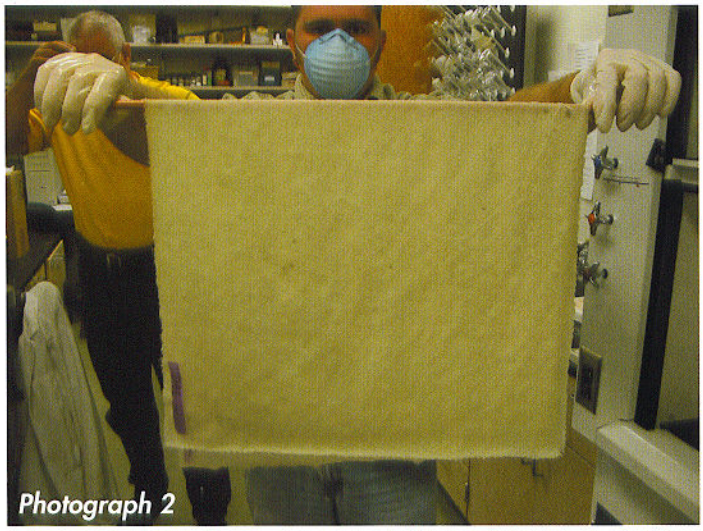
Preparation and Fabric Incubation

The experimental design evaluated the cleaning effectiveness of four fabrics (cotton, wool, polyester and silk) using four cleaning methods (perchloroethylene *aka* perc, petroleum distillates, laundry and laundry with bleach). Individual pieces of fabric (American Association of Textile Colorists and Chemists Approved), 18 inches wide and 30 inches long, were labeled and inoculated with either *Stachybotrys chartarum* (1.64×10^6 spores/milliliter) or *Aspergillus versicol*or (1.70×10^6 spores/milliliter) spores provided by P&K Microbiological Services, Inc. located in Cherry Hill, N. J. The purpose for inoculating the fabrics was to introduce a fungal species that could produce a known mycotoxin that could be quantified by a laboratory.

The spores were applied inside an aluminum foil-lined laboratory hood using a pump sprayer (RL Flowmaster, Model No. 1998, Root-Lowell Manufacturing Company). Approximately 80 milliliters (ml) of each spore suspension was used to inoculate each piece of fabric (1.31×10^8 to 1.36×10^8 total spores). Spore inoculation and incubation were conducted under non-aseptic conditions. Exposure and growth by native colonizing fungi on the test and control fabrics were unavoidable. A detailed description of the experimental methods is available from the International FabriCare Institute in Silver Spring, Md.

Three incubation chambers were prepared that contained control fabric samples (no inoculum added, native fungal species only), *S. chartarum*-inoculated fabrics and *A. versicolor* inoculated fabrics. The exterior surface of each chamber was covered with aluminum foil and the lid sealed with duct tape. The chambers were placed in

Photograph 1: Laboratory hood where the incubation chambers were stored for a nine-week period.



Photograph 2. Wool fabric removed from incubation after one-month.

Photograph 3. Hanging fabric pieces inside the incubation chamber one-month after adding diluted Gatorade (1:2 mixture).

the laboratory hood (24.5 C, constant flowing air, *Photograph 1*) and removed each week to observe evidence of fungal growth and maintain the moisture content.

One month after startup, little or no visible growth was evident on the fabrics (*Photograph 2*). At this time, a one-part Lemon-Lime Gatorade® (containing water, dextrose, fructose and ascorbic acid) to two-part water (1:2) dilution was sprayed onto the fabric (approximately 20-25 mls. per piece) to promote fungal growth. Visible fungal growth was observed two weeks later (*Photograph 3*). The incubation period extended for nine weeks.

Following incubation, all fabric samples supported light to heavy visible microbial growth and emitted a prominent moldy odor. Visual examination of the fabrics revealed the predominance of several invasive fungal species in addition to the inoculated species. The fabric samples were hand carried to and cleaned at the International FabriCare Institute (IFI)in Silver Spring, Md. The fabric samples were divided into 12 individual cleaning events.

Mycotoxin Testing

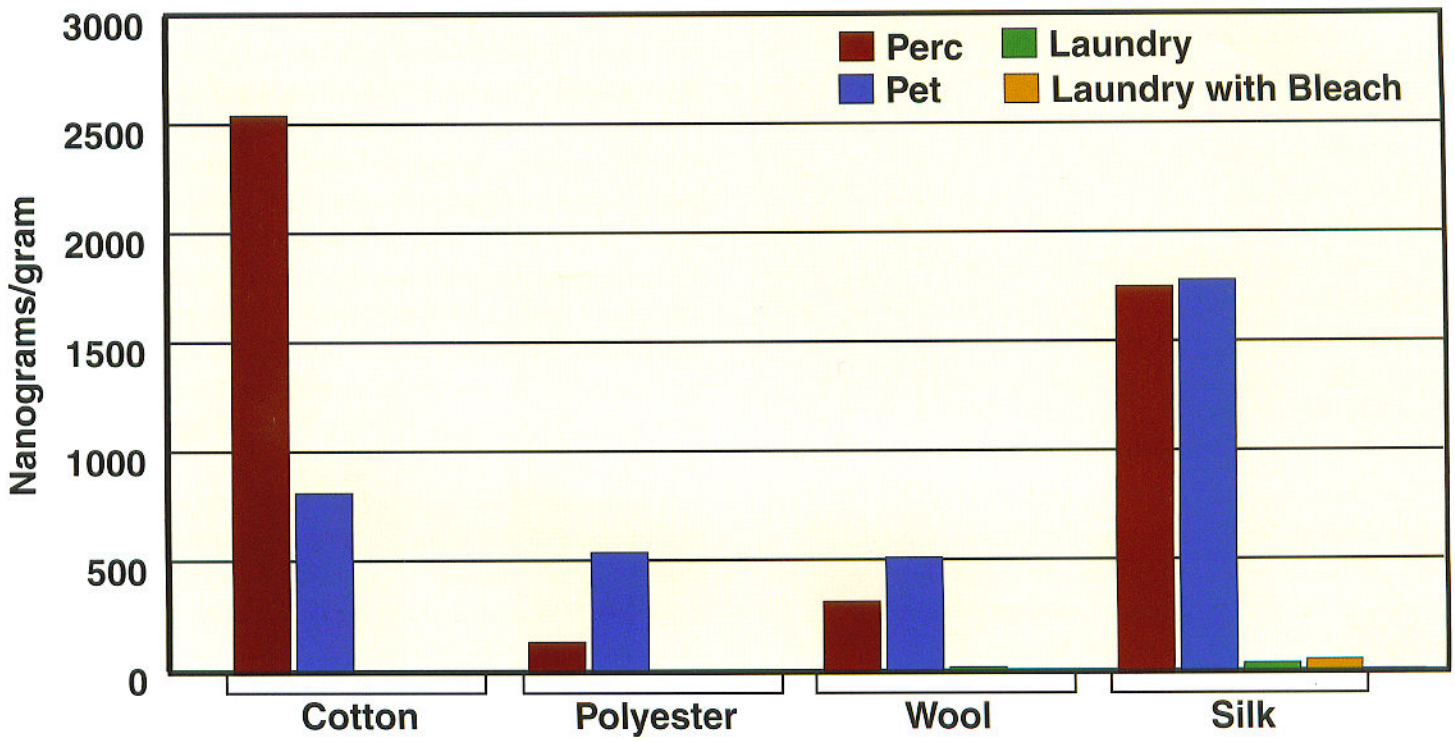
Two mycotoxins (total trichothecenes and sterigmatocystin) were selected to evaluate their removal from the mold-inoculated fabrics. Trichothecenes are a group of mycotoxins produced by *S. chartarum*; sterigmatocystin is produced by *A. versicolor*. For total trichothecenes, an ELISA based test (QuantiTox kit for trichothecenes, provided by EnviroLogix, Portland, ME) was used. Fabric samples were extracted by P&K Microbiological Services, Inc. for mycotoxin analysis using a modified procedure from the instructions provided in the test kit. The extraction was performed by adding 20 milliliters (ml) of phosphate buffered saline (PBS) to approximately 0.5 grams (g) of fabric and then vortexing for 10 minutes. The extracts were sent to appropriate laboratories for mycotoxin analyses. Sterigmatiocystin was analyzed by High Pressure Liquid Chromatography (HPLC) with MS/MS detection at Aerotech Laboratories of Phoenix, Arizona.

Results

Removal of Mycotoxins from Fabrics

Thirty-seven pieces of fabric were tested for mycotoxin content total (trichothecenes and sterigmatocystin) after cleaning. Control fabrics contained no detectable concentrations of trichothecenes and sterigmatocystin. Among the fabric samples inoculated with *A. versicolor*, none of the test and control samples results reported detectable concentrations of sterigmatocystin after cleaning. The following are the results obtained from *S. chartarum* inoculated fabrics and trichothecenes content.

TABLE 1:
RESIDUAL MYCOTOXIN CONCENTRATION IN CLEANED FABRICS



Laundry and laundry with bleach were the most effective in removing total trichothecenes among the four cleaning methods (*Table 1*). The use of perchloroethylene and petroleum distillates were less effective. Silk fabric retained detectable quantities of total trichothecenes after each cleaning method. The data indicates that total trichothecenes were removed more effectively from polyester and wool than silk. Mycotoxin (total trichothecenes) removal from cotton responded best to laundry and laundry with bleach.

The amount of fungal growth and toxin produced varied between pieces of fabric. As a consequence, the quantity of toxin present before cleaning varied and the precise percentage of removal cannot be calculated. However, based on an estimated 3×10^{-6} ng/spore toxin (total trichothecenes), the initial toxin concentration applied to each piece of fabric was approximately 390 nanograms/piece of fabric. Using the average weight (n=4) of each type of fabric (*i.e.*, polyester 26.1g, cotton 41.1g, silk 25.4g and wool 70.4 g), the amount of mycotoxin added to each piece of fabric was calculated to be:

Polyester.....14.9 nanograms/gram (ng/g)
Cotton .....9.5 ng/g
Silk.....15.4 ng/g
Wool .....5.5 ng/g

The residual total trichothecenes concentrations following cleaning with perchloroethylene and petroleum distillates ranged from 126 ng/g to 2,540 ng/g. These data indicate that conditions during incubation were favorable for mycotoxin production.

Transfer of Mycotoxins to Clean Fabrics

Total trichothecenes (8 to 60 ng/g) were transferred from mold-contaminated fabric to clean fabrics (*Table 2*). Among the four cleaning methods, laundering with bleach transferred the least amount; perchloroethylene and petroleum distillates transferred the highest concentrations of total trichothecenes to clean polyester, wool and silk fabrics. Wool appeared to be the most receptive to total trichothecenes transfer, and cotton the least receptive.

Discussion

The study results support the conclusion that the mycotoxin removal from fabric is affected by the extent of hyphal growth, fabric type and cleaning method. Fabrics that are exposed to long-term conditions (two months or more) that are favorable to fungal proliferation will be difficult to clean regardless of fabric type, cleaning method or fungal species present.

OVERVIEW

Cotton, polyester, silk and wool fabrics were inoculated (at 24.5C) with two common indoor fungi, Stachybotrys chartarum and Aspergillus versicolor to evaluate whether mycotoxins remained in fabric after they were cleaned by four methods (i.e., perchloroethylene, petroleum distillates, laundry and laundry with bleach). Each cleaning method was evaluated based on the amount of residual mycotoxins that remained in the fabric. Testing was also conducted to determine whether mycotoxins were transferred to control fabrics that were placed in the same cleaning cycle.

Fabrics cleaned with dry cleaning solvents (i.e., perchloroethylene and petroleum distillates) retained the highest residual mycotoxin concentrations. Mycotoxins (trichothecenes) were transferred from contaminated fabrics to clean fabrics when cleaned by perchloroethylene or petroleum distillates. Laundered fabrics, with or without bleach transferred the least amount of mycotoxin. Polyester was the easiest fabric from which to remove mycotoxins; silk was the most difficult.

The analytical results clearly support the need for further study. Though the experimental design was intended to promote uniform fungal growth, the extent of fungal growth on each piece of fabric varied, making absolute comparisons between the sample results difficult. Remarkably, despite these variations, common themes emerged from the analytical results and observations that make this study valuable in the interpretation of cleaning effectiveness.

Natural fibers appear to be more vulnerable to retain and adsorb mycotoxins than man-made fibers. The results prompt additional research because only one group of mycotoxins (trichothecenes) were evaluated in this research effort.

Most consumers are content that professional cleaners return their cloths pressed and smelling clean. This study greatly exceeded these qualitative criteria by using microscopic, mycological and chemical techniques for evaluation. One of the findings of this study was that mold-contaminated fabrics may pose a concern to allergy-sensitive individuals after cleaning. People who are sensitive to mycotoxins may choose to discard the visibly contaminated clothing and contents rather than attempt to have them cleaned depending on the nature of the fabrics and the cleaning method.

The diminished effectiveness in removing mycotoxins from mold-contaminated fabrics by petroleum distillates should be noted by all professional dry cleaners. Segregated cleaning of mold-contaminated clothing may be a simple way to eliminate the concern of mycotoxin transfer. It may also be prudent not to accept visibly contaminated contents.

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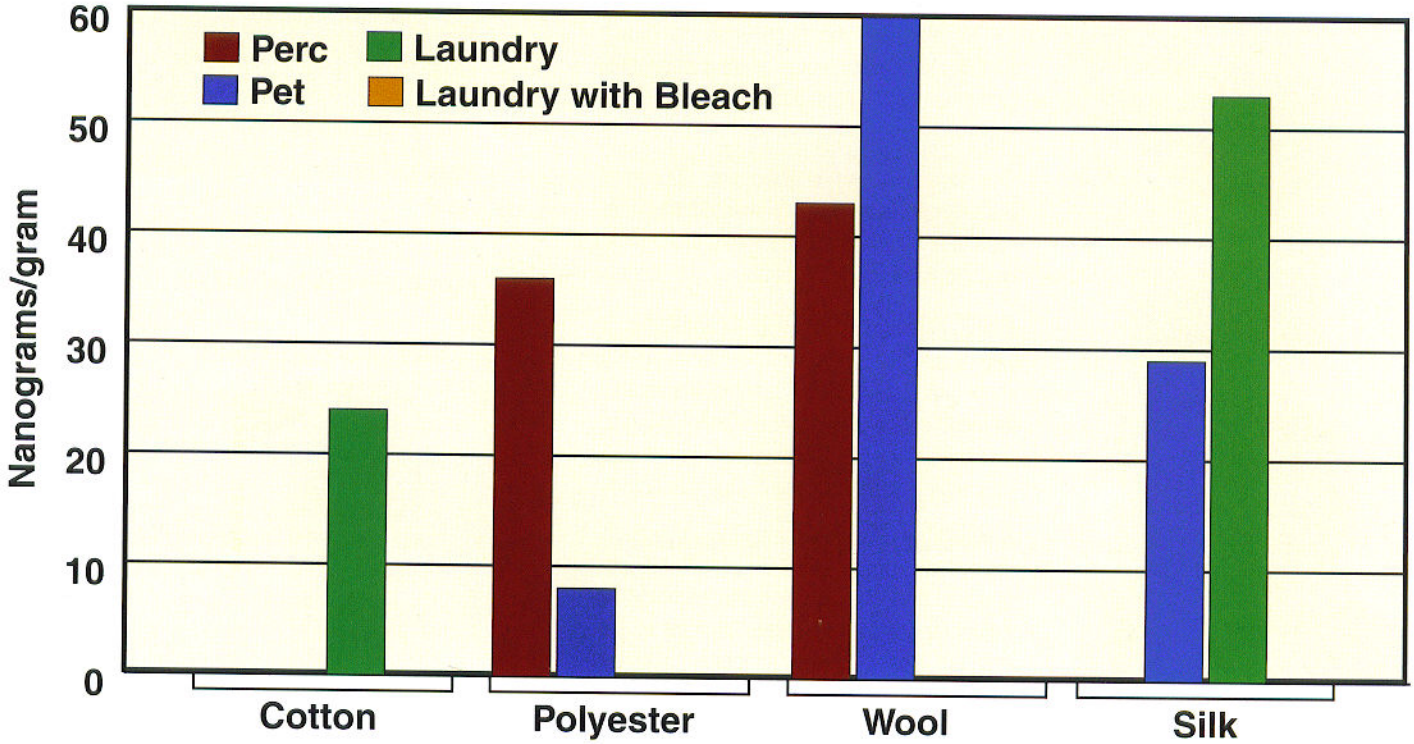
TABLE 2:
TRANSFER OF MYCOTOXIN TO CLEAN FABRICS DURING CLEANING



Two mycotoxins were used to examine whether any of four cleaning methods were effective in removing them from the test fabrics. The analysis of sterigmatocystin may have showed no detectable concentrations for several possible reasons. First, it is possible that all the cleaning methods completely removed sterigmatocystin from the fabric. Second, despite inoculating the test fabric with *A. versicolor* spores, conditions created during incubation may not have been conducive to the production of the mycotoxin. Third, despite the laboratory reporting acceptable percentage recoveries of sterigmatocystin, it is possible that the analytical method did not have a detection sensitivity for sterigmatocystin. As a result, the analytical method did not detect this mycotoxin of interest.

Laundry and laundry with bleach were the most effective in removing total trichothecenes among the four (4) cleaning methods. Overall, silk was the only fabric that retained detectable quantities of total trichothecenes after each cleaning method. The available data indicates that total trichothecenes was removed from polyester, cotton and wool more effectively than silk. SEM photos reveal the fungal hyphae attach closely to silk fibers rendering their removal difficult (*Photograph 4*).

The presence of detectable concentrations of total trichothecenes in cleaned fabrics indicates that low concentrations of some mycotoxins may be transferred during cleaning. Persons sensitized to their toxicological effects may choose to discard moldy clothes or request that their clothes be segregated when professionally cleaned. The testing results support the strategy to avoid the hamper storage of clothes for extended periods before cleaning and isolating mold-contaminated clothing for disposal.

Conclusion

Once fabrics are invaded by visible and active fungal growth, it is difficult to return the garment back to its previous condition because mycotoxins are likely to reside in the fabric after cleaning. Mold-contaminated fabrics may transfer mycotoxins to non-contaminated fabrics during cleaning. ■

Dr. Ralph E. Moon currently serves as the department manager of the Building Sciences Department at HSA Engineers & Scientists. He lectures extensively to insurance companies on the environmental consulting liability, cause and origin of water damage and building sciences.

Dr. De-Wei Li is conducting research on indoor fungi at the Connecticut Agricultural Experiment Station. He is author or co-author of 20 research papers and two books.

Dr. Chin S. Yang has worked extensively with allergists, physicians, industrial hygienists, and health professionals on microbiology-related problems and on Legionella and Legionnaires disease outbreak investigations. He has published and lectured extensively.

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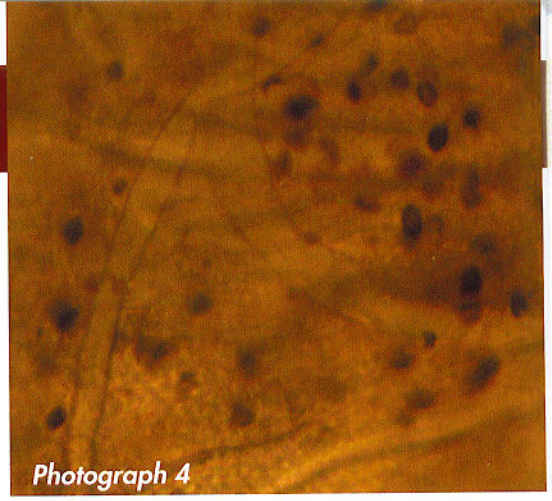

Photograph 4. Confirmation of S. chartarum spores in the fabric after incubation (200x).

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Disclaimer

This study did not attempt to define the best available technology for the removal of fungal mycotoxins. It was intended to determine how the typical dry cleaning and laundering processes work on mold removal. During the cleaning processes, we chose cationic dry cleaning detergents that are common to the industry; however, there are vast differences in detergents available in the industry. We believe that significant improvements can be made with further study of products and processes.

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