

# Effectiveness of Prime Oxide for Controlling Fungal Contamination on Building Materials



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## Abstract

**Rationale:** Mold growth on building materials can be an exposure risk for atopic individuals. The gas chlorine dioxide is an effective fungicide but is unstable. Prime Oxide (PO) is an aqueous solution containing either 0.2% or 0.5% chlorine dioxide in a stabilized form. The goal of this investigation was to determine the effects of PO on fungi commonly found growing on building materials.

**Methods:** Ceiling tile and sheetrock squares (36 cm<sup>2</sup>) were sterilized and then saturated with either sterile distilled water or various concentrations of PO. Saturated squares were inoculated with a spore suspension of one of the following fungi: *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus versicolor*, *Chaetomium globosum*, *Penicillium sp.*, and *Stachybotrys chartarum*. Cultures were incubated at room temperature for up to eight weeks. Spray applications of PO were also tested on ceiling tile squares inoculated with *Asp. fumigatus* or *S. chartarum*. Spore germination of all six species was evaluated in Sabouraud's broth with and without PO for 96 hrs.

**Results:** PO solutions containing either 0.1% or 0.2% chlorine dioxide inhibited growth of all six fungi on both ceiling tile and sheetrock squares. Spray applications were also effective for the species tested. In the germination experiments, some spore germination occurred in the medium with 0.1% chlorine dioxide ranging from <1% germination for *Alternaria* spores to 18% for *Chaetomium* spores; however, media with higher chlorine dioxide levels prevented spore germination for all species.

**Conclusions:** PO inhibited growth of fungi on sheetrock and ceiling tiles and shows possible applications for control of indoor fungal contamination.

## Introduction

- Water-damaged building materials, such as ceiling tiles and sheetrock, are prone to fungal contamination due to their high cellulose content. Fungal growth on these materials can be an exposure risk for mold sensitive individuals.
- The gas chlorine dioxide is an antimicrobial pesticide known for its disinfectant properties for the past century. This gas is an effective fungicide but requires special handling. It has been unstable in liquid and is often prepared on-site when liquid applications are needed.
- Prime Oxide (PO) is an aqueous solution containing either 0.2% or 0.5% chlorine dioxide in a stabilized form. The current project was undertaken to determine the effectiveness of PO for controlling fungal growth on building materials.

## Methods and Materials

- Prime Oxide:** Two preparations of Prime Oxide (PO) were used in these experiments: Ready To Use (PO-RTU) with 0.2% ClO<sub>2</sub> and Professional Strength (PO-PS) at 0.5% ClO<sub>2</sub>.
- Spore germination:** Spore suspensions of *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus versicolor*, *Chaetomium globosum*, *Penicillium sp.*, and *Stachybotrys chartarum* were prepared by harvesting spores from cultures grown on malt extract agar. Spore germination of all six species was evaluated in Sabouraud's broth with and without PO.
- Building material cultures:** Ceiling tile (CTS) and sheetrock (SRS) squares (36 cm<sup>2</sup>) were sterilized by autoclaving and then saturated with either sterile distilled water or various concentrations of PO. Saturated squares were inoculated with a spore suspension of test fungi and incubated at room temperature for up to 8 weeks.
- Spray application of PO:** Sterile CTS and SRS were saturated with sterile distilled water and inoculated with test fungi. When growth was visible, half the materials were sprayed with 5 sprays (3.5 ml) PO. Incubation continued for up to 10 wks.
- Viability Tests:** Sterile SRS were saturated with sterile distilled water and inoculated with *Stachybotrys chartarum* or *Aspergillus fumigatus* and incubated 2 to 4 wks. One half of the cultures were sprayed with 7 sprays (5 ml) of PO-RTU. SRS were incubated for an additional 48 hrs. Spores were harvested with a cell lifter and placed in 10 ml of sterile water. Spores were counted with a hemacytometer and percent viability was determined by dilution plating.

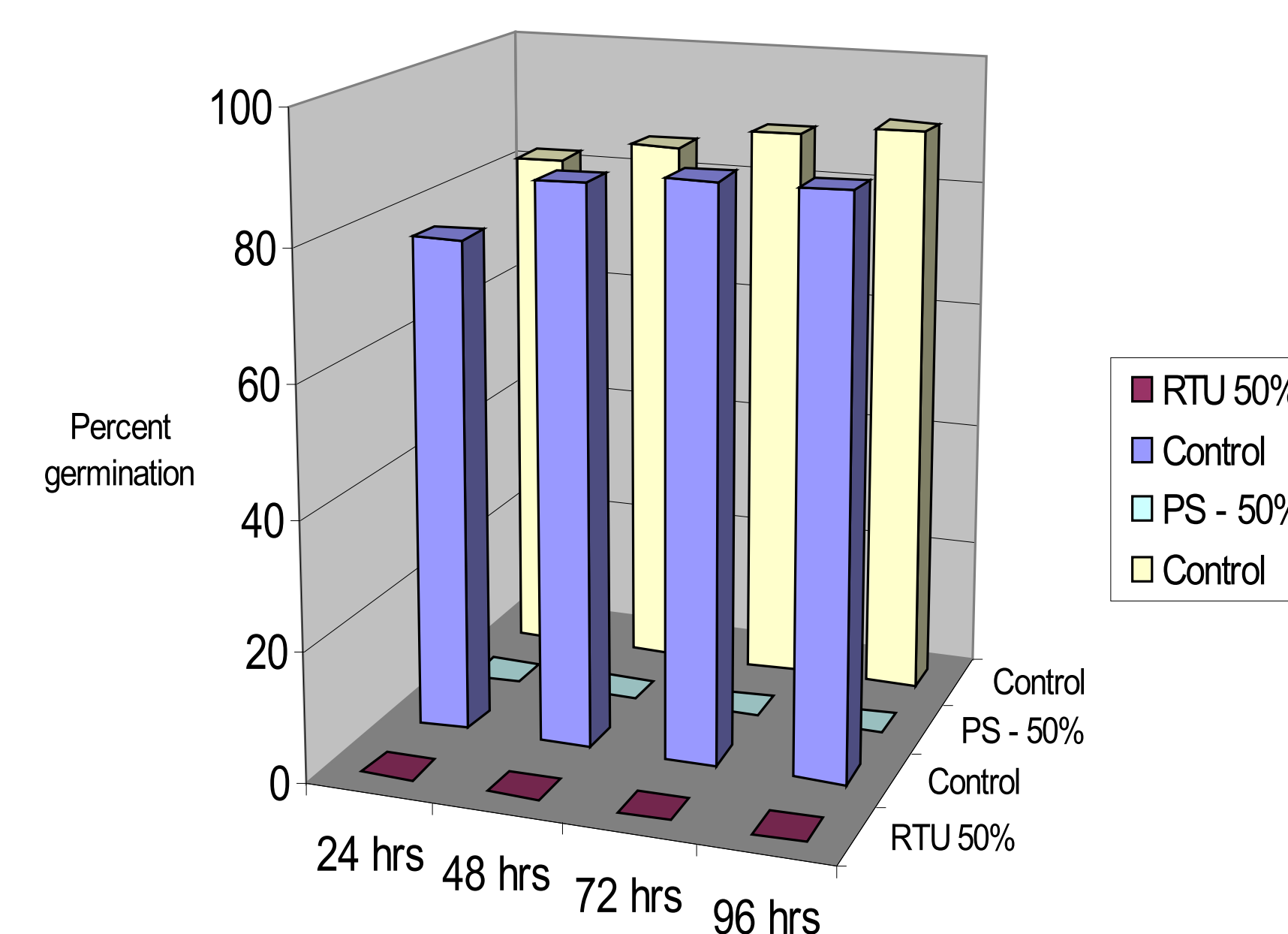
## Results

- Culture medium with 50% PO-RTU was effective in inhibiting spore germination for *Alternaria* + *Stachybotrys*, and *Penicillium* spores. *Chaetomium* spores had an 18% germination rate. The medium with 50% of PO-PS was effective in inhibiting germination for all spores.
- Pretreatment of CTS and SRS with PO-RTU was effective in preventing fungal growth. In fact, CTS were still able to inhibit fungal growth 7 months after saturation.
- Spray applications using PO-RTU on CTS and SRS with active fungal growth resulted in a resumption of fungal growth within approximately 2 wks when CTS or SRS were water saturated and not allowed to dry.
- Spray applications using PO-PS were more effective inhibiting fungal growth up to 2 months for some fungi even in water saturated conditions.
- Spray applications of PO-RTU were effective in reducing viability of *Aspergillus fumigatus* spores.

Fungus	Mean percent spore germination after 96 hours	
	Control	50% RTU
<i>Alternaria alternata</i>	>90	0
<i>Chaetomium globosum</i>	>90	18.3
<i>Stachybotrys chartarum</i>	88.3	0
<i>Penicillium sp.</i>	>90	0

Fungus	Mean percent spore germination after 96 hours	
	Control	50% PS
<i>Alternaria alternata</i>	88.3	0
<i>Aspergillus versicolor</i>	76.7	0
<i>Aspergillus fumigatus</i>	60.0	0
<i>Chaetomium globosum</i>	86.7	0
<i>Penicillium sp.</i>	>90	0
<i>Stachybotrys chartarum</i>	>90	0

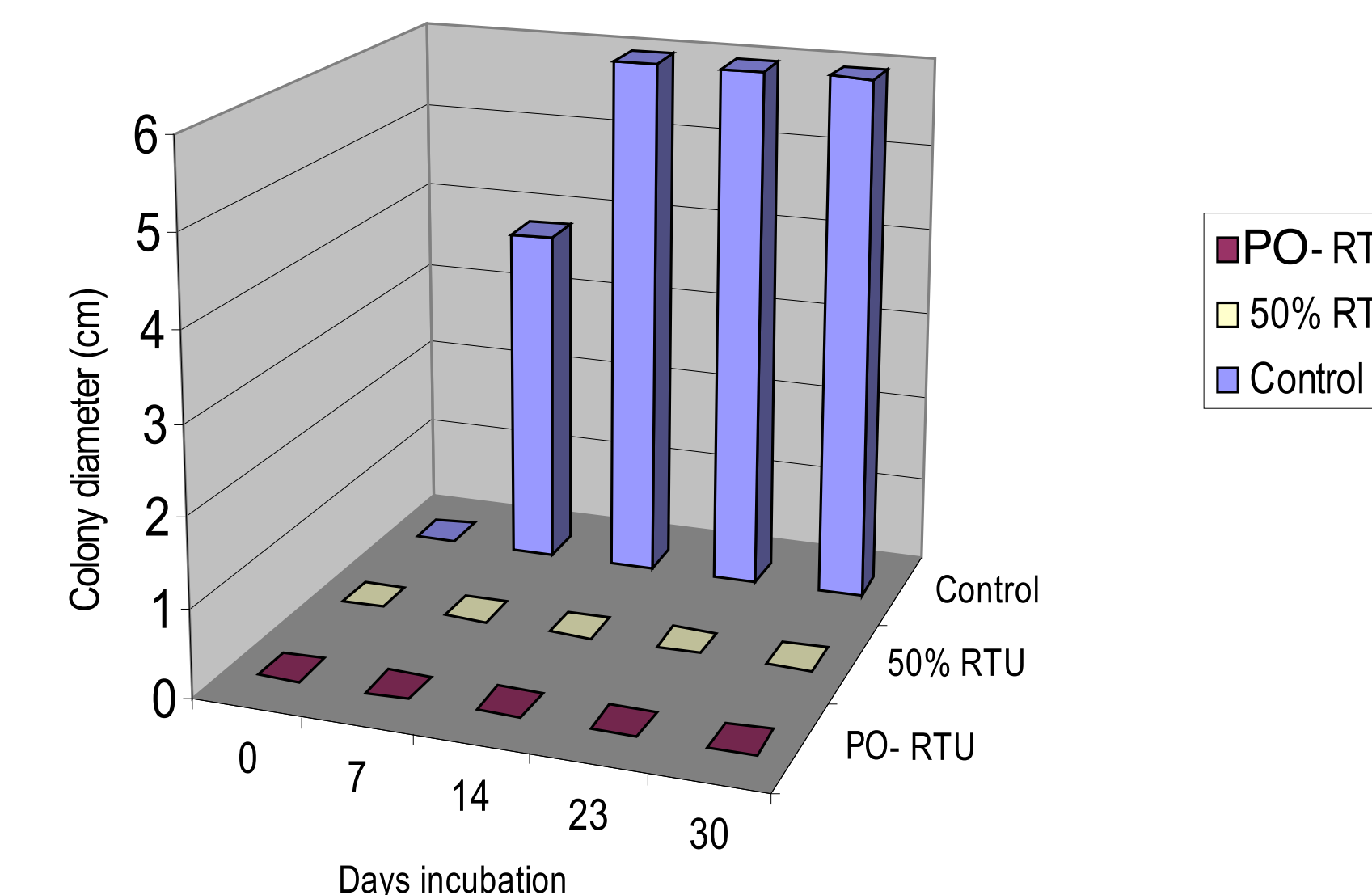
Percent germination of *Stachybotrys* spores



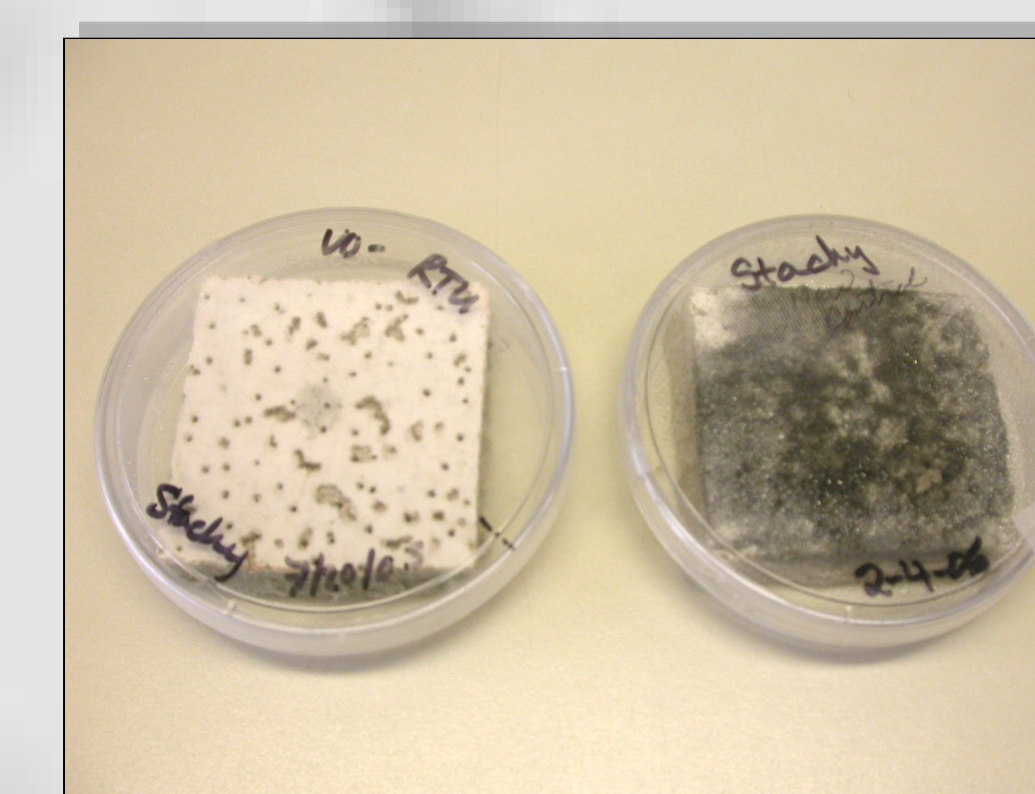
Fungi	Mean colony diameter (cm) of fungi after 30 days incubation on CTS saturated with PO or water		
	Control	PO - RTU	50% RTU
<i>Alternaria alternata</i>	>6.0	0	0
<i>Aspergillus fumigatus</i>	>6.0	0	0
<i>Aspergillus versicolor</i>	4.0	0	0
<i>Chaetomium globosum</i>	0.23*	0	0
<i>Penicillium sp.</i>	5.6	0	0
<i>Stachybotrys chartarum</i>	>6.0	0	0

\*AGrowth of *Chaetomium* on ceiling tiles could only be detected after perithecia formed. After 8 weeks perithecia were visible over an area approximately 4 cm in diameter.

Average colony diameter of *Aspergillus fumigatus* growing on ceiling tiles saturated with PO or sterile water



Fungi	Mean colony diameter (cm) of fungi after 30 days incubation on SRS saturated with PO or sterile water		
	Control	PO - RTU	50% RTU
<i>Alternaria alternata</i>	>6.0	0	0
<i>Aspergillus fumigatus</i>	>6.0	0	0
<i>Aspergillus versicolor</i>	3.6	0	0
<i>Chaetomium globosum</i>	>6.0	0	0
<i>Penicillium sp.</i>	>6.0	0	0
<i>Stachybotrys chartarum</i>	>6.0	0	0



Pretreatment of ceiling tiles with PO-RTU inhibited growth of *Stachybotrys* after 7 months following treatment. Ceiling tile saturated with PO-RTU in July 2005 was inoculated with *Stachybotrys* spore suspension on 4 Feb 2006. Control tile was inoculated at the same time.

Colony diameter of various fungi growing on saturated SRS. Treated squares were sprayed with PO-PS 2 to 3 weeks after inoculation. Final measurements and observations were made 5 to 7 weeks after spraying

	Control SRS	SRS sprayed with PO-PS
<i>Alternaria alternata</i>	6.0 cm	6.0 cm – Actively growing but less dense than controls
<i>Aspergillus fumigatus</i>	4.6 cm	No measurable colonies but one culture had small areas of growth
<i>Aspergillus versicolor</i>	5.32 cm	4.50 cm – Sprayed areas appear dead.
<i>Chaetomium globosum</i>	6.0 cm	1.85 cm – Sprayed areas appear dead.
<i>Cladosporium cladosporoides</i>	4.5 cm	3.67 cm – Sprayed areas appear dead.
<i>Penicillium sp.</i>	4.7 cm	No visible growth

Viability of *Aspergillus fumigatus* following spray application of PO-RTU

	Harvested conidia x 10 <sup>6</sup>	CFU x 10 <sup>6</sup>	Viability
Control 1	28.0	14.4	51.4%
Control 2	14.0	11.8	84.3%
Control 3	12.5	11.4	91.2%
PO-RTU Spray 1	27.5	0.34	1.24%
PO-RTU Spray 2	24.8	0.05	0.20%
PO-RTU Spray 3	36.3	0.21	0.58%

## Conclusions

- PO inhibited spore germination of test fungi
- Pretreatment with PO inhibited the growth of fungi on sheetrock and ceiling tiles
- Spray applications of PO-PS were effective in stopping fungal growth for several weeks even in saturated conditions
- More work needs to be done to determine the most effective spray application methods for PO-RTU

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