Sodium Polyborate-Based Additives on Recycled Cellulose Insulation Kill or Prevent Germination of Common Indoor Fungi

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ABSTRACT

Recent interest by the building industry about fungi and fungal-related health problems within indoor environments has spurred several ancillary industries to develop ecologically-friendly, cost-effective, safe and useful antifungal additives for building materials. Cellulose insulation, made from recycled newsprint and amended with a variety of chemical compounds to retard fire, has been touted by a variety of sources including the building industry, environmentalists, and industrial mycologists. This study reports the long-term assessment of antifungal properties of treated cellulose insulation (as a whole) and one of the most common principal active ingredients, sodium polyborate (CAS # 183290-63-3). Treated cellulose samples were challenged with a suspension containing a high concentration of fungal spores of six common fungal species. Results suggest that (a) treated cellulose insulation is sporocidal to the six species of fungi used in this study, and possibly many other fungal species; (b) this effect is long lasting (continued assessment for almost four years demonstrates little or no fungal growth on treated cellulose insulation); and, (c) using semi-permeable filters to allow only unilateral exposure to sodium polyborate, the principal active ingredient in the samples, using filters permeable to sodium polyborate, the principal active ingredient in the samples of treated cellulose, is sufficient to preclude spore germination of these same species (actually killing spores of some and perhaps killing the germinating hyphae of others).

INTRODUCTION

Persisting catastrophic flooding has focused persisting interest in humidity-based changes in indoor air quality and have spurred various constituencies to reassess, test, develop, and modify building materials that resist the detrimental effects of high relative humidity. For example, cellulose insulation, made from recycled newsprint and amended with borates (Siddiqui 1989) has garnered much attention by the building industry, environmentalists, and industrial mycologists interested in biodeterioration of building materials (Van Loo et al. 2004; Fogel and Lloyd 2002; McCleery et al. 2001). Although cellulose insulation has been used for several decades, a common concern expressed by some consumers and certain sectors of the building industry is that paper-based insulation may be susceptible to fungal growth (e.g., Hyvarinen et al. 2002). Ongoing data collection from our laboratory, however, suggests that, at least during an initial four years, a flame retardant, sodium polyborate (CAS # 183290-63-3) can reduce or eliminate fungal growth within cellulose insulation at high humidity and temperature

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(Herrera 2005), even when this cellulose is challenged with high concentrations of viable fungal spores. Continuing work with a consortium of cellulose insulation manufacturers has allowed us to systematically test whether sodium polyborate-treated cellulose insulation kills a diverse and concentrated community of fungal spores (sporocidal effect), prevents their germination (sporostatic effect) or kills germinating hyphae (hyphal toxic effect). Since this is an on-going, long-term study, the effort will determine how long these sporocidal and sporostatic effects will last. Furthermore, since sodium polyborate is the principal additive in the cellulose insulation samples tested, we assessed sodium polyborate separately for sporocidal, sporostatic or hyphal toxic properties on five different species of common indoor microfungi (*Alternaria alternata*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Penicillium chrysogenum*, and *Stachybotrys chartarum*) and a species efficient in metabolizing cellulose (*Chaetomium globosum*; Flannigan and Miller 2001; Siu 1951).

MATERIALS AND METHODS

This study is separated into two parts: Part I reports the long-term continuing assessment of the efficacy of sodium polyborate-treated cellulose insulation as compared to untreated, and shredded newsprint (control). The second part of the study (Part II) determined how much unilateral exposure to sodium polyborate can cause sporocidal/sporostatic activity.

Part I. Assessing the long-term efficacy of sodium polyborate-treated cellulose insulation.

Products tested. Five cellulose-based insulation formulations were tested: four prepared with different concentrations of sodium polyborate (FiberLite Techologies Fiber-lite®, Nu-Wool, Inc. Wallseal®, Hamilton Mfg. Inc. Thermolok®, and InCide Technologies, Inc., Pest Control® Insulation; EPA Reg. No. 44757-4), and an untreated control (not treated with any chemicals). Additional detailed information concerning the experimental procedure can be found elsewhere (Herrera 2005). During 2004, these formulations were spray-applied into five separate half-scale (1.22 X 1.22m) wall units per industry specifications (CIMA 1996). The formulation containing the lowest concentration of sodium polyborate was spray-applied first and the one containing the highest concentration was applied last (sequence: control, Nu-Wool, Fiberlite, Hamilton and InCide). Cellulose insulation was spray applied to an average depth of about 7.6cm at an average density of 48kg/m^3. The completed walls were set up on a cement slab in the (then) new outdoor Agricultural Science Laboratory building (ASL) at the Truman State University Farm (Adair County, Missouri) on 25 May, 2004. The ASL was roofed and walled only on three sides, thus protecting the wall units from rain and sun but exposing them to high ambient temperature and relative humidity throughout the summer. After the summer (124d) and since fungi are less likely to grow in cold weather, the walls were moved indoors to an air-conditioned but comparatively humid basement of Magruder Hall on the campus of Truman State University where they continued to be sampled.

Sampling. During sampling bouts, the gypsum board covering the wall units was removed and eight moisture readings (Delmhorst BD-10 moisture meter, Towaco, NJ) were taken throughout the wall units. Then, before the wall units were challenged with a fungal spore suspension, we collected "pre-challenge" (pretest) samples of cellulose from each wall unit. The detailed description of how the samples were collected is described elsewhere (Herrera 2005). Briefly, six randomly selected coordinates were used as focal sampling sites for all walls. These sampling sites (coordinates) were re-randomized for subsequent sampling bouts. Ambient T and rh (1m above floor) and T and rh readings within each unit were taken through a drilled hole in the OSB during each sampling bout. Approximately 0.25g of insulation material from each of the 6 randomly selected sampling sites was removed with sterile forceps, placed into separate, sterile, resealable plastic bags, refrigerated (5^{o}C) and processed within 12hrs.

On 31 May, 2004 after the pretest samples were collected, the cellulose in the wall units was challenged with 100ml of an atomized spore suspension consisting of about 3000 spores/ml (approximately 3 X 10^5 fungal spores in a 1% Triton X solution). Spore counts were made using a Petroff-Hauser hemacytometer. The following fungal species and approximate % of total spore load were used: *A. alternata* (Fr.) Kiessl. (1.3% of the total number of spores), *A. niger* Tiegh. (23.8%), *C. cladosporioides* (Fresen) G.A. de Vries. (11%), *P. chrysogenum* Thom (57.9%) and *S. chartarum* (Ehrenb.) S. Hughes(6%). Spore solutions of individual fungal species were prepared separately and mixed together to make a mixed-species solution, which was vortexed and immediately atomized directly onto the surface of the insulation, giving a total spore concentration of 30.27spores/cm^2 of cellulose (there are usually less than 0.3 sprores/cm^3 in most indoor environments, Herbarth et al. 2003). In total, cellulose samples were collected from each of the five wall units on 11 occasions: 31 May 2004 (pretest), 31 May (day 1 of study), 16 June (d17), 30 June (d31), 30 July (d61), 14 August (d76) and 2 October (d124), 11 November, 2005 (d547), 30 April 2006 (d717), 7 June 2007 (d1108), 1 February 2008 (d1333).

Processing of samples. a. washings--In order to obtain a more accurate understanding of both the diversity and frequency of fungal species inhabiting the insulation samples, a variation of the washing scheme described by Warcup (1955) was used (and elaborated elsewhere, Herrera 2005). About 0.05g of each sample was placed separately into a sterile wire-mesh cup and washed with a stream of pressurized distilled water and approximately 20 ml of sterile 1% Triton X solution for five min. Previous studies in our laboratory showed that this process was effective at rinsing out almost all extraneous spores while leaving actively growing fungi within cellulose fibers. b. microfungal assay--After washing the samples, small (approximately 0.5 X 0.5mm) particles of insulation material were picked out using sterile forceps and imbedded into Petri dishes filled with Malt Extract Agar (MEA) containing antibiotics (0.4g of streptomycin sulfate and 0.2g of chlortetracycline/L of media). A total of 10 pieces of insulation (5 on each of two plates containing MEA) was assessed per sample. The MEA plates were incubated at 30 ^{o}C and observed for fungal growth for a total of 10d. Microbes growing from the plates were isolated, identified and enumerated using

standard, morphological taxonomic techniques (Herrera 2005).

Part II. Assessing Sporocidal and Sporostatic Activity of Sodium Polyborate

Processing of sodium polyborate. A 1-kg lot of sodium polyborate powder was supplied on 21 July, 2004 by Jim Blasius (InCide Technologies, Phoenix, AZ) in a resealable bag. This bag was refrigerated until the powder was used (summer of 2006).

Assessment of unilateral exposure on sporocidal or sporostatic activity. The six species of fungi (and the same spore stocks) from part I (used to challenge wall units) were again used in these experiments, albeit at different approximate concentrations (*A. alternata*, 711 spores/ml (1.3% of total); *A. niger*, 18,187 (33.6%); *C. globosum* 60 (0.1%); *C. cladosporioides*, 11,072 (20.5%); *P. chrysogenum*, 23,917 (44.2%); *S. chartarum*, 150 (0.3%).

The effect of unilateral exposure on viability was assessed using four standard Petri dishes containing media amended with 5% sodium polyborate (w/w; by mixing sodium polyborate powder into media previously autoclaved and molten, but cooled to 50^{o}C). Plates 1 and 2 contained MEA and water agar (WA), respectively. A sterile cellophane filter (Osmonics Inc., 0.45micron pore size, Minnetonka, MN) was then placed on the media's surface and 10, 1µl aliquots of a fungal spore suspension were pipetted at the surface and margins of this filter (Figure 1). The filter is permeable to sodium polyborate (and nutrients), but does not allow fungal spores to pass. Plates 3 and 4 were prepared in a similar fashion except that the spore suspensions contained Triton X (a surfactant). Because concurrent assessment of Triton X suggested that it did not influence spore viability (for example, by facilitating the penetration of sodium polyborate into spores or denaturing fungal proteins), we opted to pool data describing spore viability of plates 3 and 4 with that of plates 1 and 2. An additional plate not containing boron was used as a positive control for each of the fungal species.

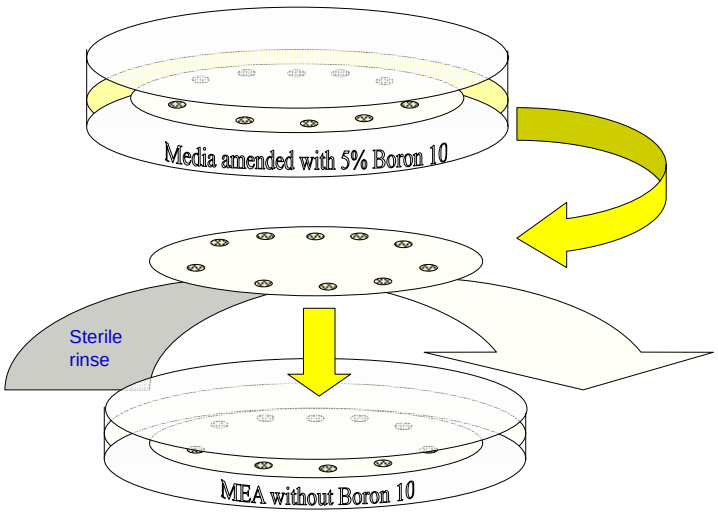

FIGURE 1. Schematic diagram describing sampling and processing protocol for part II

of study assessing sporocidal activity of sodium polyborate-treated insulation.

After the spores were exposed to the 5% sodium polyborate for 7d, the filter was removed and cleaned free of any boron residue by rinsing the underside of the filter with a gentle stream of sterile distilled water. Each filter then was placed on separate MEA plates (without boron) and incubated at 30^{o}C for 10d. The number and diameter of fungal colonies emerging from the second set of plates was assessed and compared to their corresponding control plates and among the six fungal species used.

RESULTS

Part I. Assessing the long-term efficacy of sodium polyborate-treated cellulose insulation.

A total of 3159 cellulose insulation particles were assessed as of 2 February 2008. Of these, a total of 330 harbored fungi (10.4%), nearly all of these particles were untreated controls (253 of 330 or 76.6%) or particles obtained soon after the walls were challenged with spores (Figure 2). After the first couple of days of the study, however, all of the formulations harbored nearly equal and very low levels of fungal growth on their cellulose. Although the fungal challenge increased the percentage rate of growth of both the control and treated insulation, it was obvious that the control samples had significantly higher viable fungal loads during the pretest (day 0). In subsequent sampling over the next 3.5 years, fungal loads appeared to track relative humidity values (particularly for the control samples; Figure 3).

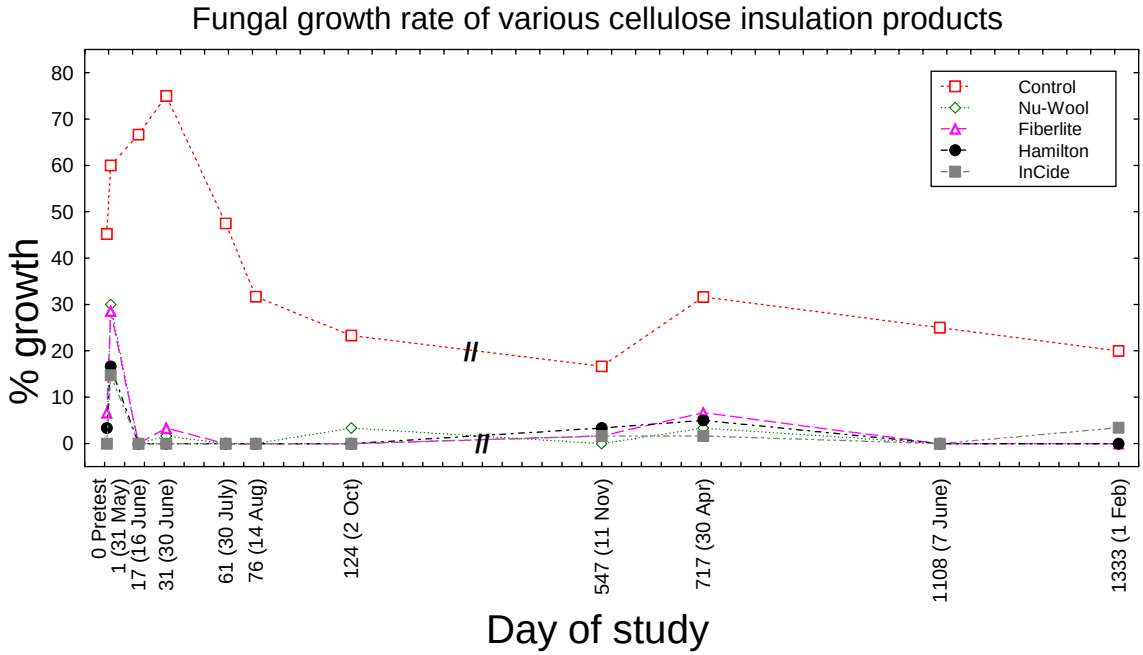

FIGURE 2. % of fungal growth on the five formulations of cellulose insulation tested.

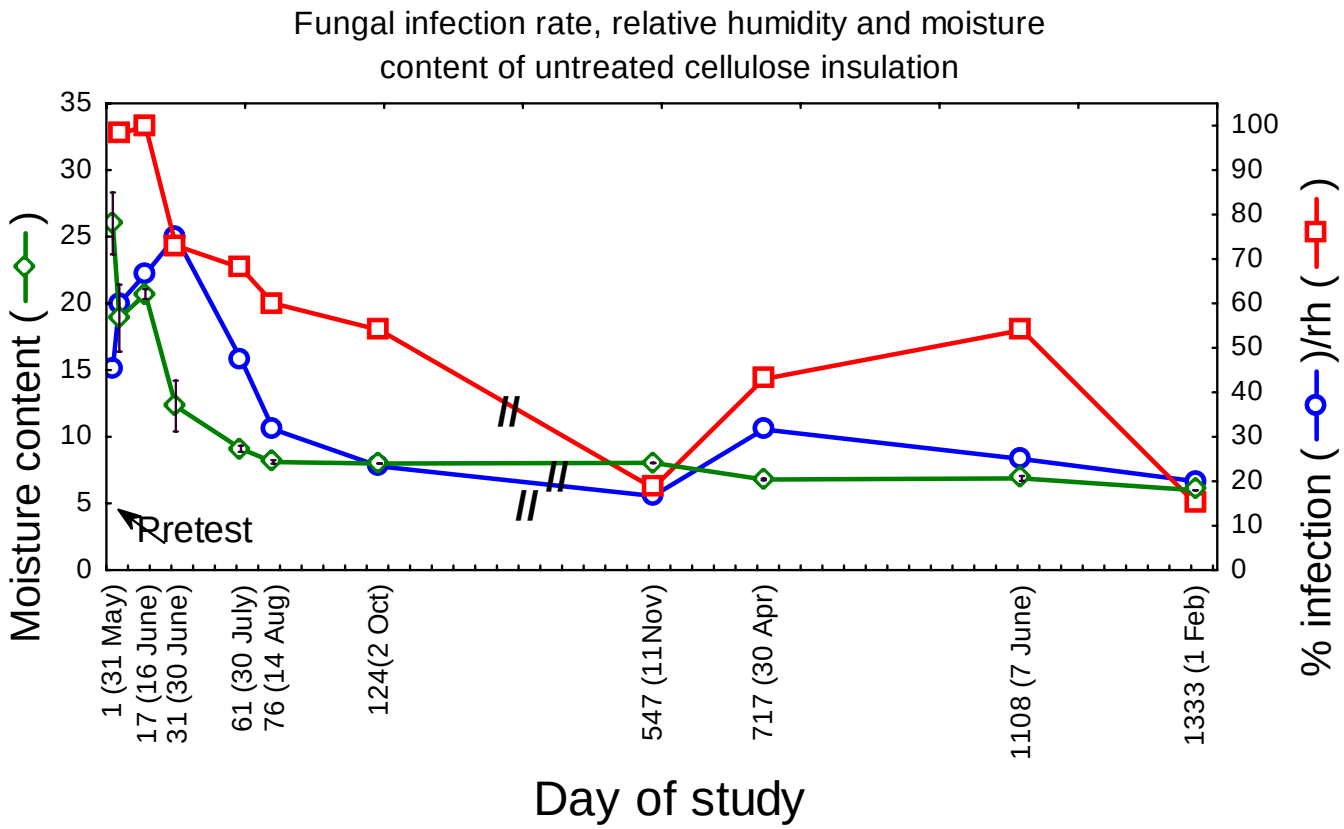

FIGURE 3. Mean (±SE) moisture content (diamonds; n = 8 measurements per sampling date), relative humidity (squares) and percentage of fungal growth (circles) measured on untreated (control) cellulose insulation during the study.

Part II. Assessing sporocidal and sporostatic activity of sodium polyborate.

Assessment of unilateral exposure on sporocidal or sporostatic activity. Spores from all fungal species did not grow on filter paper while on MEA or WA amended with 5% sodium polyborate. Once transferred to MEA without boron, however, the responses for each of the six species varied. Aliquots containing spores of *A. alternata* and *C. cladosporioides* did not exhibit growth, while aliquots from *S. chartarum* and *P. chrysogenum* exhibited growth on MEA only when exposed to sodium polyborate in WA. Spores exposed to sodium polyborate in WA were nearly twice as likely to grow compared to spores exposed to sodium polyborate in MEA. A post-hoc Wilcoxon Signed Rank test (Zar 1984) exhibited marginal significance, suggesting that this was generally true ($Z = 1.63$, $P = 0.05$), and our data (Table 1) suggests that it was particularly true for *P. chrysogenum* and *S. chartarum*. Aliquots of *A. niger* and *C. globosum* exhibited growth regardless of whether they were exposed to sodium polyborate in MEA or WA (Table 1), though compared to controls, the development (diameter) of the colonies was protracted by about two to three days

TABLE 1. Percent of fungal spore inoculations exhibiting growth on Malt Extract Agar (MEA) without sodium polyborate after 10d. Twenty, 1µl aliquots of a suspension containing spores of six fungal species were placed on filter paper and unilaterally exposed to 5% sodium polyborate in MEA or Water Agar (WA) for 7d before being transferred to MEA without sodium polyborate.

Species	% inoculations growing on MEA after pre-exposure to sodium polyborate in MEA	% inoculations growing on MEA after pre-exposure to sodium polyborate in WA	% of inoculations growing on MEA after sodium polyborate pre-exposure (row totals)
A. alternata	0%	0%	0% (0/40)
A. niger	90%	100%	95% (38/40)
C. globosum	100%	100%	100% (40/40)
C. cladosporioides	0%	0%	0% (0/40)
P. chrysogenum	0%	100%	50% (20/40)
S. chartarum	0%	90%	45% (18/40)
Totals	32% (38/120)	65% (78/120)	48% (116/240)

Effect of spore concentration. Spore concentration did not obviously influence viability rates of spores. For example, aliquots containing the spores of *C. globosum* (harboring the lowest spore concentration of the six species used) were most likely to exhibit growth (as high as those mixtures containing spores of *A. niger* and *P. chrysogenum*, which had the highest spore concentrations; Table 1).

DISCUSSION

Our results clearly indicate that, with this protocol, cellulose insulation treated with formulations that include sodium polyborate are very effective at killing and/or preventing germination of high concentrations of common indoor fungi for extended periods under optimal conditions for fungal growth. Treated cellulose harbored very few viable spores or actively growing mycelia even after 3.5yrs of high relative humidity and moderate temperatures. This was true regardless of whether the treated insulation had been washed free of spores (Herrera et al. 2007). In other words, though the treated insulation was sprayed with artificially high concentrations of fungal spores known to grow on paper, few (or no) viable spores were found within treated insulation. Conversely, untreated paper homologs readily grew different species of microfungi (primarily *Aspergillus niger*; Herrera et al. 2007).

Sodium polyborate, the principal component of all tested formulations was effective at preventing fungal growth even when fungal spores were exposed only on one side. Even when the spores were removed from unilateral contact with sodium polyborate and placed on media (MEA) containing no borates, only two of the six species grew, though this growth appeared to be protracted compared to their controls. Interestingly, some species (*P. chrysogenum* and *S. chartarum*) only exhibited growth if exposure to sodium polyborate occurred on WA, a media that does not provide carbohydrates and, consequently, does not encourage spore germination. Although sodium polyborate's sporostatic effect held for all fungal species, supplying a carbohydrate source may elicit germination of the spores and allow sodium polyborate to induce a hyphal toxic effect on *P. chrysogenum* and *S. chartarum*. This is reasonable

In sum, the results lead us to conclude that the presence of sodium polyborate within cellulose insulation prevents fungal growth. At the concentration tested (5%), sodium polyborate seems to manifest this inhibition by preventing spore germination of all species, killing spores of some species, and possibly killing the germinating (and metabolically active) spores of others. Although the actual concentration of sodium polyborate used in the formulations is proprietary, it is typically higher than 20% (by weight) and much higher than the 5% concentrations used in the media of this study. Consequently, this difference in sodium polyborate concentrations may be responsible for the differences in sporocidal activity reported on treated insulation product and our study using unilateral exposure to 5% sodium polyborate.

The efficacy of the sporocidal properties exhibited by treated cellulose insulation may be, in part, also accounted for by other additives. Many of these products contain other chemicals that, although making up a smaller proportion of the active compounds, may represent a measurable effect on the level of sporocidal activity exhibited by the product as a whole. If true, it is likely that the effectiveness of the treated insulation is potentiated by these compounds acting as complementary chemical units. Indeed, Clausen and Yang (2004) suggest as much in their work that showed anti-fungal effectiveness was improved when some borates were included in “multicomponent” mixtures. This complementation of antifungals is generally true for other antibiotics that kill a different suite of microbes via distinct mechanisms (e.g., Shinn and Lim 2004).

The fact that spores of some species exposed to sodium polyborate on different media differed in their response is a bit perplexing. In particular, although germination and growth was inhibited by 5% sodium polyborate regardless of media and conditions, unilateral exposure on solid MEA plates appears to have a sporocidal effect while placing spores in MEA broth seemed to have a sporostatic effect or in the case of *C. globosum*, little to no affect at all. Perhaps the difference in response lies in the availability of water and its corresponding changes in osmolarity in aqueous solutions or in the differential effects of sodium polyborate’s mechanism of action in solution. It is also possible that the spores that did not succumb to the sporocidal effect of sodium polyborate did not interact with the chemical sufficiently long enough. Perhaps, *P. chrysogenum* and *C. globosum* spores are more resistant to the effects of sodium polyborate and take longer to kill. Although the chemical mechanism for sodium polyborate antifungal activity remains unknown, several investigators as early as 1971 (Philipp and Bender), suggested that numerous borate compounds are effective as reversible inhibitors of serine proteases (Irving et al. 2003). If true, inhibition of some members of this diverse family of enzymes may prevent the polymerization of chitin, an important fungal cell wall constituent and disturb some of the disparate metabolic pathways. The diverse targets and mechanisms of boron containing compounds may help explain why sodium polyborate is sporocidal to some species and sporostatic or hyphal toxic to others.

CONCLUSIONS

In conclusion, our results indicate that treated cellulose insulation is sporocidal for nearly all species of fungi tested and that the principal active ingredient, sodium polyborate, plays a predominant role in the product's antifungal properties. Boron's diverse mechanisms of activity seem to determine whether those antifungal properties are manifested as sporocidal, sporostatic or hyphal toxic effects on any one species of fungi. Although our long-term studies are continuing, the results of nearly four years of data suggest that the building industry should consider treated sodium polyborate as an insulation material even when the construction materials are challenged with high fungal loads.

ACKNOWLEDGEMENTS

Some of the processing of the samples was made by Audrey Kerr, Niba Nchutu, Melvin Omodon, Clayton Dillavou, Greg Malnassy and David Chappell. An anonymous reviewer improved the contents of the manuscript. Treated cellulose insulation and their paper homologs were supplied by Hamilton Manufacturing, Inc. (Twin Falls, ID), Nu-Wool Company, Inc., (Jenison, MI), Fiberlite Technologies, Inc. (Joplin, MO). Portions of this study were supported by a consortium of cellulose manufacturers, headed by InCide Technologies, Inc. (Phoenix, AZ) and by Truman State University.

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