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Pre-contamination of new gypsum wallboard with potentially harmful fungal species

Abstract Gypsum wallboard is a popular building material, but is also very frequently overgrown by *Stachybotrys chartarum* after severe and/or undetected water damage. The purpose of this study was to determine whether *Stachybotrys* and other fungi frequently isolated from wet gypsum wallboard are already present in the panels directly from the factory. Surface-disinfected gypsum disks were wetted with sterile water, sealed, and incubated for 70 days. The results showed that *Neosartorya hiratsukae* (≡ *Aspergillus hiratsukae*) was the most dominant fungus on the gypsum wallboard followed by *Chaetomium globosum* and *Stachybotrys chartarum*. Our results suggest that these three fungal species are already embedded in the materials, presumably in the paper/carton layer surrounding the gypsum core, before the panels reach the retailers/building site.

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Key words: Drywall; *Chaetomium*; *Neosartorya*; *Stachybotrys*; Surface disinfection; Recycled paper/cardboard.

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Practical Implications

This study shows that wet gypsum wallboard, regardless of type or brand, is prone to fungal growth because fungi are already incorporated into the material during production. This study demonstrates the importance of securing buildings constructed with high amounts of gypsum wallboard against water damage and high humidity. It also shows that industry has a major task ahead in ensuring that fungal spores are not built into their products and that a hazard analysis and critical control points approach, as used in the food industry, would seem to be a good strategy to ensure a minimal fungal contamination of new building materials.

Introduction

Gypsum wallboard (drywall or plasterboard) is used extensively in both new builds and renovation projects throughout the world because it is cheap, easy to work with, and fire resistant. It is, however, also prone to fungal growth if exposed to high humidity or water ingress. Growth of the filamentous fungus *Stachybotrys chartarum* is particularly associated with wet gypsum wallboard worldwide (Flannigan and Miller, 2011), and research has shown that in Danish water-damaged buildings, *S. chartarum* occurs on 25% of the gypsum wallboard samples, compared to only 4% in other samples examined (Andersen et al., 2011). Other filamentous fungal genera, such as *Penicillium*, *Chaetomium*, and *Ulocladium*, have also been associated with wet gypsum wallboard (Flannigan and Miller, 2011; Gravesen et al., 1999), but these associations are less significant (Andersen et al., 2011).

Many filamentous indoor fungi can produce species-specific bioactive metabolites during growth. *Chaetomium globosum* produces chaetoglobosins and *Penicillium chrysogenum* produces PR toxin, roquefortines, and penicillins, whereas *S. chartarum* is known for its production of macrocyclic trichothecenes and atranones (Nielsen et al., 1998; Samson et al., 2010). Some of these metabolites (roquefortine A, chaetoglobosin A, and roridin E (a macrocyclic trichothecene) (Polizzi et al., 2009)) as well as fungal cell wall components (β-glucans (Rand and Miller, 2011)) have been detected in indoor air, and fungal growth in buildings is therefore problematic, as some of these compounds may have a negative impact on the health and well-being of the occupants (Carey et al., 2012;

Mussalo-Rauhamaa et al., 2010; Nikulin et al., 1997; Rosenblum Lichtenstein et al., 2015); especially, *S. chartarum* is of concern, because it is also able to produce hemolytical proteins (Nayak et al., 2013) and macrocyclic trichothecenes that have previously been associated with a number of animal and human health problems, for example, leukopenia in horses, sheep, and rabbits (Drobotko, 1944; Forgacs et al., 1958; Harrach et al., 1983; Jarvis et al., 1986) and pulmonary hemorrhage and hemosiderosis in infants in Cleveland (Etzel, 2007; Jarvis et al., 1998).

S. chartarum can be found on moldy cellulose-rich materials, such as hay, plant debris, enriched soil, and paper (Domsch et al., 2007; Ellis, 1971), but is rarely detected in air (Baxter et al., 2005; Viegas et al., 2014), because the spores are borne in sticky slime heads (Samson et al., 2010). Detection of viable spores in air samples is usually only possible when large areas (>m^2) covered with *Stachybotrys* growth are disturbed and sampled close-up (Dill et al., 1997; Tiffany and Bader, 2000). *C. globosum* can be found in similar cellulose-rich habitats as *S. chartarum* (Domsch et al., 2007) and is equally difficult to get airborne because its spores are borne in ascomata formed in cracks and cavities (von Arx et al., 1986). In nature, both species are thought to have beetles, ants, and mites as dispersal vectors (von Arx et al., 1986; McGinnis, 2007).

Common belief has it that fungal growth in damp/wet buildings happens because fungal spores from outside drift indoors and randomly start growing on any available material. This may be true for genera, such as *Aspergillus, Cladosporium*, and *Penicillium*, which are readily airborne and much more common in the environment (Samson et al., 2010). In the case of *Chaetomium* and *Stachybotrys* on gypsum wallboard, however, the lack of viable airborne spores in the outer environment and the overrepresentation of *Stachybotrys* on wet gypsum wallboard point to another source than outside air/environment as the origin of contamination. Price and Ahearn (1999) found *Chaetomium* and *Stachybotrys* in their untreated gypsum samples and noticed in a passing remark: 'These species were presumably part of the inherent bioburden on the gypsum wallboard following manufacturing and storage'. The purpose of this study was therefore to determine whether *Stachybotrys* and other indoor fungi are already present in unused and undamaged gypsum wallboard.

Materials and methods

Building materials, treatment, and incubation

Thirteen different panels of gypsum wallboard (900/1200 × 2400 × 13 mm) were bought in four different *do it yourself* (DIY) shops around Copenhagen over a period of six months. Two different brands and three different types of gypsum wallboard were used in this study (see Table 1). Each panel was divided into four sections (900/1200 × 600 mm), and three circular disks (70 mm in diameter) were cut from each section (twelve disks in total per panel). Each set of 12 disks was surface-disinfected for 30–45 s by submerging one disk at a time in 1000 ml of 96% ethanol and gently rubbing both surfaces. Three additional sets of twelve disks from panel no. 9 were also surface-disinfected in Rodalon (according to the directions for use), household bleach (according to the directions for use), or sterile water following the same procedure as for ethanol.

After surface disinfection, the disks were placed in a rack and allowed to air-dry to constant weight, usually overnight, in a sterile laminar airflow (LAF) bench (23–25°C and 30–35% RH). Each disk was placed aseptically into an empty, sterile Petri dish (plastic, 90 mm in diameter) and weighed. Sterile water was added to the Petri dish to reach an approximate 23% water content (w/w of the constant weight) and weighed again. Each Petri dish was then sealed with Parafilm and incubated at room temperature (22–23°C) for 70 days. Disks of the first nine panels were evaluated qualitatively for fungal growth every other week (the presence or absence of each genus on a disk), through the lid without disturbing the disk, using a stereomicroscope. Fungal colonies were identified to genus level based on their morphology. Disks of the last four panels were also evaluated on days 3 and 7. After 70 days, the Petri dishes were weighed again and opened and representative colonies were isolated for species identification using morphology and metabolite profiling according to Andersen and Nissen (2000), Andersen et al. (2002, 2003), Samson et al. (2007, 2010), and Wang et al. (2016).

Results

Analysis of the thirteen different panels of gypsum wallboard showed that there was fungal growth of one

Table 1 Type and origin (anonymized) of the gypsum wallboard panels used in this study

Panel no.	Type of wallboard	Brand	Outlet	Purchase date
1	Fire resistant	A	DTU[a]	14-01-2015
2	Fire resistant	A	DIY-1	20-01-2015
3	Fire resistant	B	DIY-2	17-03-2015
4	Moisture resistant	B	DIY-2	17-03-2015
5	Fire resistant spacer	A	DIY-1	23-03-2015
6	Fire resistant	A	DIY-3	30-04-2015
7	Moisture resistant	A	DIY-3	30-04-2015
8	Fire resistant	B	DIY-4	30-04-2015
9	Moisture resistant	B	DIY-4	30-04-2015
10	Fire resistant	A	DIY-1	13-07-2015
11	Regular	A	DIY-1	13-07-2015
12	Fire resistant spacer	A	DIY-1	13-07-2015
13	Fire resistant	B	DIY-2	13-07-2015

[a]Clean, unused surplus panel from a building site at the Technical University of Denmark.

or more fungal species, on either the face, the reverse, or on both, on all tested panels (Table 2). Panel 9 (moisture resistant, brand B) had the highest total fungal count (64 fungal counts on 12 disks), while panel 3 (fire resistant, brand B) had the lowest (21 fungal counts on 12 disks). The analyses also showed that there were no major differences between brands or between same types of panel purchased from different DIY outlets. During the 70-day incubation period, fungal growth and dissemination were followed and recorded using a stereomicroscope. Only six genera, namely *Neosartorya*, *Aspergillus*, *Chaetomium*, *Penicillium*, *Cladosporium,* and *Stachybotrys*, reoccurred on three or more of the thirteen panels (Table 2). *Alternaria*, *Botrytis*, *Phoma,* and *Ulocladium* were found on only one panel and one or two disks, while *Harzia, Paecilomyces,* and *Pochonia* were found only once.

Fungal identification

At day 70, the seals of the Petri dishes were broken and representative colonies were isolated and identified to species. The most abundant fungal species found on gypsum wallboard was *Neosartorya hiratsukae*. This fungus was found on all thirteen panels (100%) and on most disks, both face and reverse. It appeared first on the reverse of the disks, but within days it was also visible on the face. The fungus had covered the whole disk with small (100–230 μm in diameter) white ascomata (cleistothecia) (Figure 1a/d) in 3–7 days after first appearance. Only on panel 5 was *N. hiratsukae* more abundant on the face than on the reverse (Table 2). The most abundant *Aspergillus* turned out to be *A. hiratsukae*, which is the asexual state of *N. hiratsukae* (i.e., the same organism as *N. hiratsukae*) and was found around the edge of the disks and constituted most of the recorded '*Aspergillus'* shown in Table 2. *C. globosum* was the second most abundant species, found on eleven panels (85%) and was equally common on both face and reverse. It took ca. 14 days after onset for the fungus to cover the whole surface of the disk with large (300-500 μm in diameter) dark ascomata (perithecia) with green curly hair (Figure 1b/e). Both *P. chrysogenum* and *Cladosporium cladosporioides* showed the same pattern by occurring mostly on the face of nine (69%) and eight (62%) panels, respectively, and only as discrete, slow-growing colonies that stopped expanding after 14–21 days (Figure 1b). *S. chartarum* (both chemotypes) was found on seven (54%) of the thirteen panels and would cover the whole surface of the disk within 7 days after onset with black clusters of slime heads (Figure 1c/f). *Alternaria infectoria*, *Aspergillus versicolor,* and *Cladosporium sphaerospermum* were found on only one panel and on one or two disks, while *Aspergillus fumigatus*, *Chaetomium elatum,* and *Penicillium polonicum* were found only once.

Surface disinfection

After the emergence of *N. hiratsukae* ascomata on the first 5 panels, it was speculated whether the ethanol was triggering the growth of this fungus or whether it could be present in the ethanol. Therefore, two other surface disinfectants, household bleach and Rodalon, and autoclaved water were also used on subsamples of panel 9. Analysis of the three subsamples showed similar results compared to ethanol disinfection (Table 3). With water and bleach, the ascomata of *N. hiratsukae* appeared just as rapidly (14 days) as they did with ethanol, whereas the Rodalon treatment delayed the ascomata production by ca. 7 days. The subsample washed in autoclaved water did not show any additional fungal growth, and neither bleach nor Rodalon hampered the growth of *S. chartarum*. Table 3 also shows that the water content of the disks decreased slightly from 22.5% to 20.8% on average

Table 2 The number of disks with fungal growth on face (F) and reverse (R) from 13 different gypsum wallboard panels. Twelve disks from each panel were surface-disinfected with ethanol, wetted with sterile water, and incubated for 70 days

		Neosartorya		*Aspergillus*		*Chaetomium*		*Cladosporium*		*Penicillium*		*Stachybotrys*	
Panel no.	% Water Day 1	F	R	F	R	F	R	F	R	F	R	F	R
1	21.6	4	10	–	1	7	8	3	–	5	1	7	7
2	21.3	8	12	–	–	5	3	2	–	3	1	1	1
3	27.0	2	9	–	–	–	–	–	–	1	1	4	4
4	21.8	8	8	7	5	4	4	3	1	6	4	1	2
5	24.2	11	1	3	1	2	3	2	–	2	–	10	11
6	22.8	12	12	2	–	2	1	1	–	–	–	–	–
7	23.2	12	12	–	1	2	1	–	–	–	1	–	–
8	23.6	3	7	1	–	1	1	9	–	1	–	–	–
9	23.0	12	12	12	12	3	4	–	–	8	–	1	–
10	23.2	12	11	12	–	2	1	–	–	–	–	1	1
11	22.5	12	12	4	–	–	–	4	–	–	–	–	–
12	22.5	12	12	4	2	1	–	–	–	–	–	–	–
13	23.0	6	12	1	2	1	2	8	–	3	1	–	–

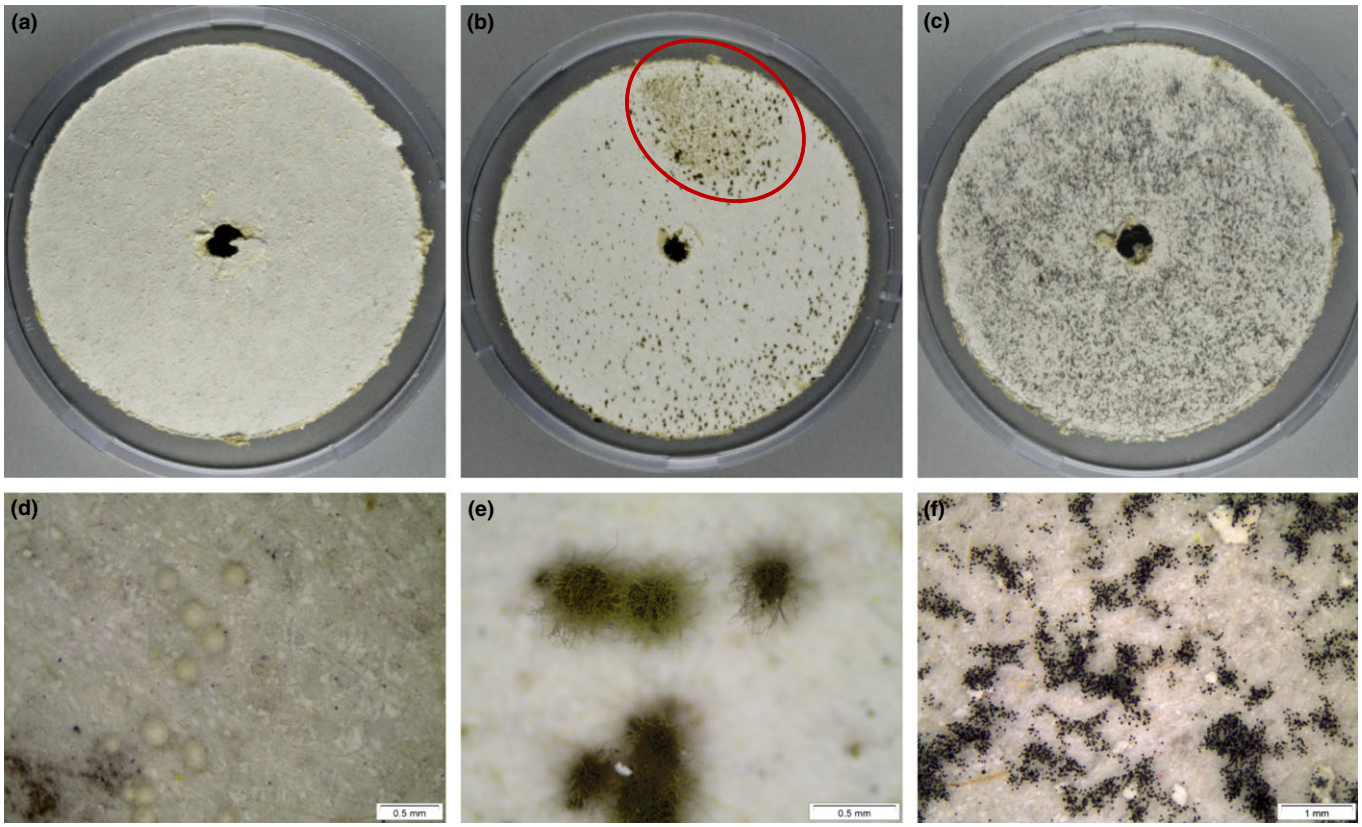

Fig. 1 Gypsum disks (face up) with fungal growth after 70 days. a/d: *Neosartorya hiratsukae* (panel 3); b/e: *Chaetomium globosum* (panel 1) with *Penicillium chrysogenum* encircled; c/f: *Stachybotrys chartarum* (panel 3).

during the 70-day-long incubation period despite several layers of Parafilm around the edge of the Petri dishes.

Onset of fungal growth

The last four panels (panels 10–13) were also examined on day 3 and day 7 to see how quickly the fungi could germinate, grow, and sporulate. Figure 2 shows the onset of sporulation for each genus and the end time where no new fungal colonies appear. The first undifferentiated hyphal growth was evident on day 3 (graph not shown) and conidial heads of *A. hiratsukae* were visible after only 7 days of incubation, whereas *N. hiratsukae* ascomata started to appear after 14 days. Also, *C. cladosporioides* and *P. chrysogenum* were visible after 14 days with conidiophores and the first chains of conidia. The first slime heads of *S. chartarum* were visible after 21 days, while *C. globosum* ascomata were visible after 28 days (panels 6–9, result not shown). No new colonies appeared after 42–45 days.

Discussion

The results of this study showed that gypsum wallboard is already contaminated with fungal spores before the panels reach their end-users, because the same fungal species, *Neosartorya hiratsukae* (≡ *Aspergillus hiratsukae*), *Chaetomium globosum,* and *Stachybotrys chartarum*, were found repeatedly in the paper/cardboard of all surface-disinfected samples irrespective of type, brand, or outlet.

N. hiratsukae, which is an uncommon fungus in the environment, has to our knowledge never been reported on gypsum wallboard before. It has previously been isolated from soil, fruit juice, and indoor air (Samson et al., 2007). *N. hiratsukae* is also reported to be pathogenic to humans (Guarro et al., 2002) and to produce avenaciolide (Samson et al., 2007). One reason for occupants and surveyors not to realize the growth of this fungus could be that the small white ascomata are evenly distributed across the white paper surface of the gypsum wallboard and therefore not readily visible to the naked eye (Figure 1a) and difficult to see without a slanted light source on the stereomicroscope. The pale green anamorphic state of the fungus, *A. hiratsukae*, is produced only sparingly on gypsum wallboard. During the 70-day incubation period, it was noted how the ascomata slowly disintegrated and released vast numbers of ascospores. This suggests that *N. hiratsukae* spores (both live and dead) and microparticles from the ascomata can easily become airborne and constitute a health risk in build-

Table 3 The number of disks with fungal growth on face (F) and reverse (R) from panel 9 after treatment with four different surface disinfectants. Twelve disks for each treatment were surface-disinfected, wetted with sterile water, and incubated for 70 days

Surface disinfectant	% Water (w/w)		*Neosartorya*		*Aspergillus*		*Chaetomium*		*Cladosporium*		*Penicillium*		*Stachybotrys*	
	Day 1	Day 70	F	R	F	R	F	R	F	R	F	R	F	R
Ethanol	23.0	21.6	12	12	12	12	3	4	–	–	8	–	1	–
Water	21.6	19.9	12	12	12	12	3	5	2	–	10	9	–	–
Bleach	22.3	21.0	12	12	12	12	3	2	–	–	6	–	2	2
Rodalon	23.1	20.7	12	12	12	–	2	–	–	–	7	–	–	1

ings with water-damaged gypsum wallboard without the knowledge of the occupants.

A limited number of research studies have been published on the occurrence of *C. globosum* in water-damaged buildings, and even fewer have reported *C. globosum* on paper and gypsum wallboard (Flannigan and Miller, 2011; Jerusik, 2010; Price and Ahearn, 1999). One reason for not detecting *C. globosum*, even though it is very conspicuous on gypsum wallboard when mature (Figure 1b), may be its long lag phase on both gypsum and laboratory media. Furthermore, samples from water-damaged buildings often contain other fungi (e.g., *Penicillium* spp.) that grow much faster on laboratory media and thereby obscure any growth of *C. globosum*.

The association between *S. chartarum* and gypsum wallboard, on the other hand, is well documented, and it was therefore expected that *S. chartarum* would be the dominant fungus. Still, more than 50% of our panels were contaminated with *S. chartarum*, which is more than some studies report (Andersen et al., 2011; Gravesen et al., 1999), but less than the 60–77% Flannigan and Miller (2011) found in their study. If our results are representative for a panel (1 of 12 disks is contaminated with one viable *S. chartarum* spore), it would suggest that a whole panel would be contaminated with 40–60 viable spores depending on the size of the panel (ca. 20 spores/m^2). This corresponds well to our field observations in buildings with severe water damage. There, we have seen simultaneous outgrowth of numerous discrete *Stachybotrys* colonies on the same gypsum panel, indicating that *Stachybotrys* spores are distributed throughout the material. On our most contaminated sample, panel 5, where 10 disks were contaminated, the same estimation would give approximately 200 viable *S. chartarum* spores per m^2. This combined with the rapid growth rate on gypsum wallboard (covering a disk in less than a week) might explain why *S. chartarum* can dominate a water-damaged building so quickly.

Most gypsum wallboard manufacturers advertise that they use recycled materials in their production. Recycled paper/cardboard is often collected in big bails at the recycling center and stored under less than optimal conditions (Jerusik, 2010) where bails are exposed to rain, soil, and insects, which results in high microbial loads (Betz and Cerny, 1999). *N. hiratsukae*, as well as *C. globosum* and *S. chartarum,* can survive at temperatures around 40°C (Domsch et al., 2007; Samson et al., 2007), and even if a small percentage of the fungal spores survive pulping and drying, there could be invisible fungal growth on the gypsum wallboard after as little as 7 days after major water damage. However, because it is not known where in the process the different fungi enter or what their contamination and survival rates are, further research into the specific production methods of the raw materials is needed.

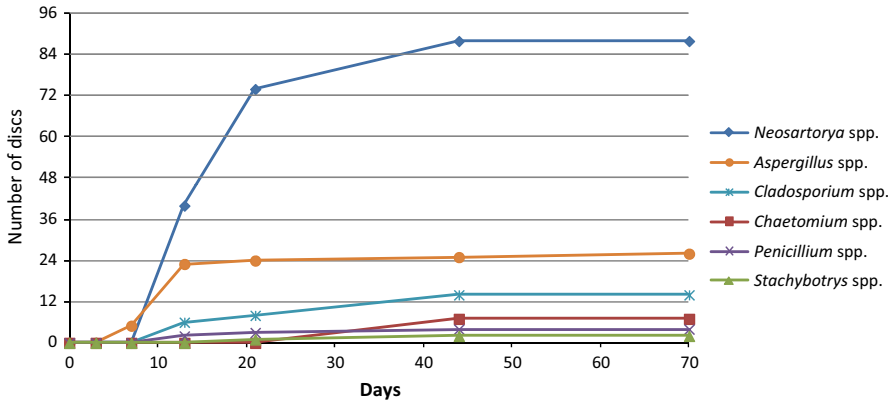

Fig. 2 Time (days) until first appearance of fungal growth/sporulation on disks (both face and reverse) from panels 10–13 (n = 96).

One approach to safer gypsum wallboard is for the manufacturers to use hazard analysis and critical control points (HACCP), which is a system used by the food industry for decades to ensure food safety, but other types of industry are increasingly using HACCP as a competitive parameter.

Ongoing chemical analyses of the disks at our department will show which fungal metabolites (e.g., avenaciolide, chaetoglobosins, atranones, and satratoxins) these inbuilt fungal contaminants are able to produce during their growth on the gypsum wallboards, which is consistent with previous findings on *Stachybotrys* spp. (Nielsen et al., 1998). Also, a rapid detection method using DNA sequencing directly on the paper/cardboard surface is being developed at our department.

Conclusions

The results of this work show that gypsum wallboard is contaminated with *Neosartorya, Chaetomium*, and *Stachybotrys* and suggest that the spores of these fungi are embedded in the paper/cardboard surrounding the gypsum core. Even though the manufacturers do not market their gypsum wallboard as 'sterile' or 'fungal free' most consumers trust that there are no potentially harmful fungi in their building materials. Because the growth of these fungi can result in large quantities of microparticles and bioactive compounds, which may be released into the indoor air after water damage, there is a need for manufacturers to undertake a stricter quality control of their raw materials and finished products. However, even the best efforts of the manufacturers would be rendered futile if proper/correct shipping, handling, and storage are not equally strictly controlled. During storage, construction, and occupancy, gypsum wallboard must be kept dry, clean, and undamaged to provide safe and healthy buildings.

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