Evaluation of the Antimicrobial Activity of Water-Borne and Polyurethane Solvent-Borne Coating Materials for Wooden Furniture According to Common and Newly Invented Testing Methods

Hana Polášková,* and Daniela Tesařová

The strong antimicrobial effect of a dry-film of acrylic water-borne coating materials for wooden furniture was proven in this study. The reduction of more than 4 logarithmic orders of bacteria after 24 h of contact with a coating material was confirmed for *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterococcus faecalis.* An antifungal effect against *Aureobasidium pullulans*, *Penicillium funiculosum*, *Cladosporium cladosporoides*, and *Aspergillus niger* was discovered. Although water-borne coating systems are considered to be environmentally friendly, the antimicrobial activity of in-can preservatives remained effective even after the formation of a dry-film. The release of antimicrobial substances (preservatives) from the finished dry-film can be dangerous, especially in the case of wooden toys or products for children. An antibacterial effect from the polyurethane solvent-borne coatings was not proven. The agar diffusion plate test, JIS Z 2801 (2012) and EN 15457 (2015) were used for the evaluation of the antimicrobial effect under wet conditions. Moreover, a new test method for the evaluation of the antibacterial activity was created, which better reflects real contamination under dry conditions. This method is based on the contamination of talc particles by bacterial spores of *Bacillus subtilis*, which simulates dust particles contaminated by airborne bacteria. An antibacterial effect for water-borne and polyurethane solvent-borne coating materials tested according to this new dry method was not proven.

Keywords: Water-borne coating materials; Polyurethane solvent-borne coating materials; Antimicrobial effect; Biocides; In-can preservatives; Wet bacterial contamination; Dry bacterial contamination

*Contact information: Department of Furniture, Design and Habitat, Mendel University in Brno, Brno, the Czech Republic; *Corresponding author: xpolask8@node.mendelu.cz*

INTRODUCTION

There has been a trend over the last few years towards the use of environmentally friendly, healthy, and safe coating materials for interior wooden furniture. Producers are making an effort to substitute solvent-borne coating materials that have high VOC levels with high solid or water-borne coating materials that have low VOC levels (Désor *et al*. 1999; Rijckaert *et al*. 2001; Athawale and Nimbalkar 2011). Water-borne coating materials are generally considered to be environmentally friendly and safe, but they also provide the appropriate moisture conditions for microbial growth and contain many components that could act as a source of nutrients for microorganisms (Bravery 1988; Makarewicz *et al*. 2011). During the manufacture and storage of coating materials,

bacteria, molds, and yeasts can cause viscosity loss, gassing, malodor, pH drift, visible surface growth, loss of corrosion inhibition, and other undesirable effects (Davison 2003). After application, the dry film may be also infected with microorganisms, resulting in the disfigurement and breakdown of the coating (Gillatt 1998). If a water-borne coating material in a wet-state is unprotected by biocides, which are effective against bacteria and fungi, then it is prone to microbial contamination and spoilage (Downey 1995). Solvent-borne coating materials do not typically require this type of protection. This means that although water-borne coating materials do not contain harmful solvents, they must be treated with biocides because of in-can preservatives (Lindner 2001; Wallström and Hoffmann 2001; Köhler *et al*. 2011; ECHA 2016). The most common biocides are isothiazolone (IT) and formaldehyde donor (FD) biocides (Davison 2003). This article studies these coating materials, which are frequently used for wooden furniture, from a microbiological point of view.

Regulation (EU) No 528/2012 (2012) distinguishes between "biocidal products" and "treated articles". Simply, a "biocidal product" is any substance used with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on any harmful organism by any means other than mere physical or mechanical actions (Regulation (EU) No 528/2012 2012). If a product has only been treated with a biocide, but does not have a primary biocidal function, it is a "treated article" (ECHA 2016). Biocides used as in-can preservatives for water-borne coating materials fall into the Product-type 6, which are preservatives for products during storage, according to Regulation (EU) No 528/2012 (2012). The water-borne coating material is considered to be a "treated article", which means that a biocidal function should not be expected.

Recently, the antimicrobial treatment of various products has become a global concern. Many studies have focused on the improvement of the antimicrobial characteristics of daily-used products or equipment. This treatment should be safe ecologically for humans. Water-borne coating materials are considered to be non-toxic, environmentally friendly, and safe. They are commonly used as finishing for wooden toys and products for children, which are subject to Directive 2009/48/EC (2009). The task of this study was to find answers for the following questions: *How safe are water-borne coating materials? Does the antimicrobial effect of in-can preservatives remain active after the formation of a dry-film?*

Miscellaneous microbial life in the air can cause serious health problems. Ubiquitous airborne microbes and cell fragments combined with byproducts of cellular metabolites in liquid droplets are a natural part of the air. Additionally, there are viruses, bacteria, and fungi floating on dust particles in the air (Schleibinger *et al*. 2004; Stetzenbach *et al*. 2004). Pathogenic microorganisms can cause infectious diseases, respiratory disorders, and other allergic, inflammatory, and toxic responses (Buttner *et al*. 2002; Stetzenbach *et al*. 2004). Germs such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* (MRSA), *Clostridium difficile*, and *Acinetobacter* spp. are among the most dangerous germs that are responsible for hospital-acquired infections (HAIs) (Ducel *et al*. 2002; Dancer 2014; Khan *et al*. 2015). Therefore, the antimicrobial treatment of furniture, which will inhibit the microbes in the air of interior spaces, is necessary.

There are two basic methods for testing the antimicrobial activity of various surfaces. The first is based on the determination of whether or not antimicrobial

substances are being released from the sample to the surroundings, and the second is based on the incubation of microorganisms on the sample surface and the comparison of the number of microorganisms at the beginning of testing to the number after a certain incubation time (usually 24 h). Both methods are performed under wet conditions, which does not reflect real situations very well. Furniture is usually contaminated by dust, other dry particles, or small liquid droplets that evaporate very quickly, all of which can carry microorganisms. Unfortunately, no testing method simulating these dry conditions has been previously published. In this article, a method for testing samples under dry conditions will be described.

EXPERIMENTAL

Materials

Coating materials

Commercially available acrylic water-borne and polyurethane solvent-borne (two components) glossy coating materials for wooden furniture were used. The coating materials were applied in one layer on filter paper, done according to the producer's instructions, and were dried 7 days prior to the experiments under standard atmospheric pressure with a relative humidity of 45 ± 5% and temperature of 23 ± 2 °C.

Tested germs

The following tested bacterial germs were used: *Escherichia coli* (CCM 4517), *Staphylococcus aureus* (CCM 4516), *Enterococcus faecalis* (CCM 4224), *Pseudomonas aeruginosa* (CCM 1961), *Bacillus atrophaeus* (*subtilis*) (CCM 4624), and *Klebsiella pneumoniae* (CNCTC 6120). The following tested fungal strains were used: *Aspergillus niger* (CCM 8155), *Aureobasidium pullulans* (CCM 8182), *Penicillium funiculosum* (CCM F-161), and *Cladosporium cladosporoides* (CCM F-348). The microorganisms were obtained from the National Institute of Public Health (Praha, the Czech Republic) and Czech Collection of Microorganisms (Brno, the Czech Republic).

Media for cultivation and dilution of microorganisms

Nutrient broth, plate count agar (PCA) for bacterial cultivation, malt agar for fungal cultivation, and tryptone water for dilution (HiMedia Laboratoires Pvt. Ltd., Mumbai, India) were all used. Each medium was sterilized in an autoclave at 121 °C for 15 min. The agar plates were prepared according to standard microbiological techniques.

Test Standards

The qualitative agar diffusion plate test, a routine testing method for the determination of diffusion of antibacterial substances from the samples to the agar, and quantitative JIS Z 2801 (2012) were used for the evaluation of antibacterial activity. The standard EN 15457 (2015) was used for the determination of the resistance to fungal growth.

Methods

Antibacterial activity - agar diffusion method

Five mL of agar contaminated with various tested bacteria were spread evenly over the sterile agar surface. Afterwards, the samples were placed on the contaminated agar, and the Petri dishes were cultivated for 24 h. After that, the growth of bacteria under and around the sample was evaluated.

Antibacterial activity - JIS Z 2801 (2012)

The bacterial suspension was directly inoculated on the sample surface. The tested bacteria (inoculum) were adjusted to a concentration of 1 x 10^5 to 3 x 10^5 CFU/mL by a McFarland nephelometer (Erba Lachema s.r.o., Brno, the Czech Republic). Nutrient broth, after it had been diluted 500 times (for *Escherichia coli* and *Klebsiella pneumoniae*) or 10 times (for *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*) with water, was used for the preparation of the inoculum. The inoculum, 0.15 mL, was pipetted onto all of the tested samples (size 30 x 30 mm), which were then placed in sterile Petri dishes and covered with polyethylene foil (size 25 x 25 mm). Immediately after inoculation, the tested samples were washed with 10 mL of tryptone water. The number of bacteria (at 0 h) was determined by the plate count method, *i.e.* a method in which the number of bacteria is calculated by counting the number of colonies according to a ten-fold serial dilution. One mL of each dilution was pipetted into a Petri dish, and approximately 15 mL of PCA were poured into the dishes and mixed. The same procedure was performed after incubating the samples for 24 h at 35 °C in a thermostat. Each sample was tested three times. The average values were given in the final tables of the results. After incubation of the Petri dishes for 24 to 48 h, the number of bacteria in 1 mL (c_B in CFU/mL) was counted. The final number of bacteria (M) was calculated according to Eq. 1:

$$M = c_B \cdot 10 \quad (1)$$

where M is the number of bacteria per specimen (CFU), c_B is the bacterial concentration obtained by the plate count method (CFU/mL), and 10 is the volume of the shake-out medium (mL). All values of uncertainty were calculated with the assumption of a normal distribution (P = 68.3%).

Antibacterial activity - action against dry microbial contamination

Talc particles (Koltex Color s.r.o., Mnichovo Hradiště, the Czech Republic, 95% of particles sizes are < 15 µm) were contaminated by bacterial spores of *Bacillus subtilis*. Washed bacterial spores prepared in a 96% ethanol solution were added to sterile talc. The final concentration of the contaminated talc was 5 x 10^5 to 8 x 10^5 spores per 1 g of talc. The testing inoculum was prepared by adding 0.4 g of contaminated talc to 5 mL of 96% ethanol. This ethanol talc suspension was diluted 10 times in ethanol. Of this diluted suspension, 0.2 mL were used as a testing inoculum, which was homogenously spread over the sample (30 x 30 mm). The ethanol was then allowed to evaporate. Afterwards, the samples were incubated for 24 h at 25 °C. The number of bacteria (at 0 and 24 h) was determined by the plate count method, like for the previous method. All values of uncertainty were calculated with the assumption of a normal distribution (P = 68.3%).

Antifungal activity - EN 15457 (2015)

The fungal spore suspension was inoculated directly on the specimens and agar. The tested fungal spore suspension in distilled water was adjusted to a concentration of 10^6 spores/mL by a Bürker counting chamber (Paul Marienfeld GmbH & Co.KG, Lauda-Königshofen, Germany). Of each fungal suspension, 0.4 mL were homogenously spread on the agar, on which tested samples had been placed. The Petri dishes were incubated at 29 °C for 7, 14, and 21 d. After this time, the growth of fungi on and around the sample was evaluated.

RESULTS AND DISCUSSION

Evaluation of the Antibacterial Activity

Agar diffusion plate test

The antibacterial testing of the acrylic water-borne coating material itself, without any other applied antimicrobial treatment, showed very interesting results. The agar diffusion plate test (Table 1) confirmed a good antibacterial effect against *Klebsiella pneumoniae* and *Staphylococcus aureus*. However, an antibacterial effect on Gram-negative *Pseudomonas aeruginosa*, one of the most dangerous pathogens responsible for HAIs, was not proven. For comparison purposes, the polyurethane solvent-borne coating material was tested under the same conditions. An antibacterial effect was not proven for this coating. Additionally, a water-borne coating material without applied in-can preservatives used for storage of the final product was tested. An antibacterial effect was also not determined for this coating according to this testing method.

Table 1. Antibacterial Activity of the Coating Materials Tested According to the Agar Diffusion Plate Test

	Water-borne coating material	Water-borne coating material without in-can preservatives	Polyurethane solvent-borne coating material
	Bacterial growth assessment under the sample		
Klebsiella pneumoniae CNCTC 6120	Only some restricted colonies No IZ* Good effect	Moderate Insufficient effect	Moderate Insufficient effect
Stapyhylococcus aureus CCM 4516	None No IZ* Good effect	Moderate Insufficient effect	Moderate Insufficient effect
Pseudomonas aeruginosa CCM 1961	Slight Insufficient effect	Moderate Insufficient effect	Moderate Insufficient effect
* IZ – inhibition zone around the sample			

The agar diffusion plate test is a qualitative method. When bacteria do not grow under or around the sample, which is the inhibition zone (IZ) that appears around the tested sample, it means that antimicrobial substances are being released from the sample. This method is used in practice, *e.g.* for the evaluation of the activity of antibiotics. These

results indicated that the in-can preservatives of the water-borne coating materials still have an antibacterial effect under wet conditions, even after the formation of a dry-film.

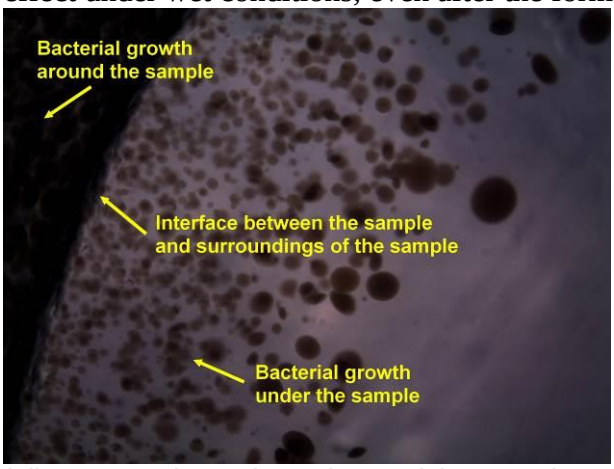

Fig. 1. Growth of *Klebsiella pneumoniae* under and around the water-borne coating material (under microscope, magnification 40x)

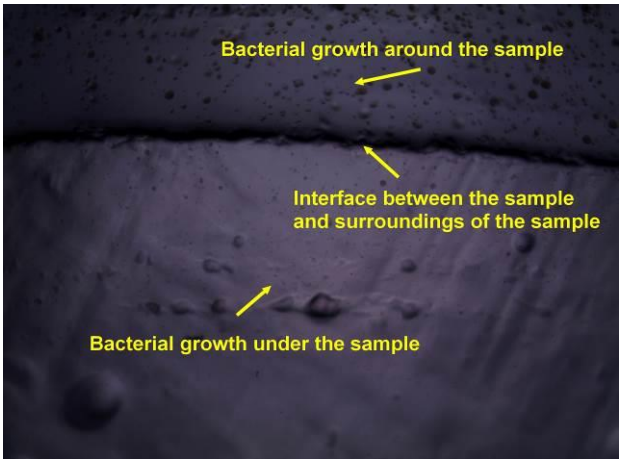

Fig. 2. Growth of *Staphylococcus aureus* under and around the water-borne coating material (under microscope, magnification 40x)

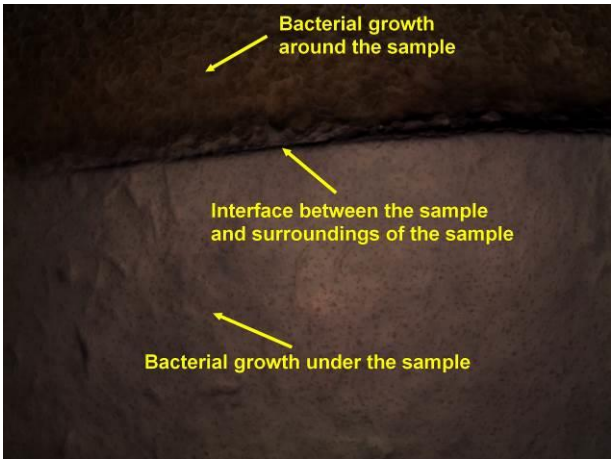

Fig. 3. Growth of *Pseudomonas aeruginosa* under and around the water-borne coating material (under microscope, magnification 40x)

JIS Z 2801 (2012)

The water-borne and polyurethane solvent-borne coating materials were tested according to the quantitative Japanese test method JIS Z 2801 (2012) in order to find out how bacteria survive on the surface of the samples under wet conditions. This method simulates a situation in which the samples are covered with contaminated liquid. All of the testing methods used in laboratories for the evaluation of antimicrobial activity use a bacterial liquid suspension to inoculate samples with bacteria. This is not a very realistic method because the liquid enables antimicrobial substances to be in better contact with the bacteria. In reality, it is not usual for the liquid medium to be between the surface and microbes for several hours.

Table 2. Results of the Antibacterial Activity of the Water-Borne Coating Material Tested According to JIS Z 2801 (2012)

	Pseudomonas aeruginosa CCM 1961	*Klebsiella pneumoniae* CNCTC 6120	*Escherichia coli* CCM 3954	*Staphylococcus aureus* CCM 4516	*Enterococcus faecalis* CCM 4224
CFU/sample 0 h	$(2.8\pm0.6) \times 10^4$	$(2.5\pm0.4) \times 10^4$	$(2.1\pm0.2) \times 10^4$	$(1.9\pm0.2) \times 10^4$	$(1.7\pm0.2) \times 10^4$
CFU/sample after 24 h	< 10	< 10	< 10	< 10	< 10

Table 3. Results of the Antibacterial Activity of the Water-Borne Coating Material Without In-Can Preservatives Tested According to JIS Z 2801 (2012)

	Pseudomonas aeruginosa CCM 1961	*Klebsiella pneumoniae* CNCTC 6120	*Escherichia coli* CCM 3954	*Staphylococcus aureus* CCM 4516	*Enterococcus faecalis* CCM 4224
CFU/sample 0 h	$(2.6\pm0.5) \times 10^4$	$(2.6\pm0.3) \times 10^4$	$(2.3\pm0.2) \times 10^4$	$(1.7\pm0.2) \times 10^4$	$(1.6\pm0.2) \times 10^4$
CFU/sample after 24 h	< 10	< 10	< 10	< 10	< 10

Table 4. Results of the Antibacterial Activity of the Polyurethane Solvent-Borne Coating Material Tested According to JIS Z 2801 (2012)

	Pseudomonas aeruginosa CCM 1961	*Klebsiella pneumoniae* CNCTC 6120	*Escherichia coli* CCM 3954	*Staphylococcus aureus* CCM 4516	*Enterococcus faecalis* CCM 4224
CFU/sample 0 h	$(2.7\pm0.6) \times 10^4$	$(2.3\pm0.5) \times 10^4$	$(2.5\pm0.5) \times 10^4$	$(2.0\pm0.2) \times 10^4$	$(1.6\pm0.2) \times 10^4$
CFU/sample after 24 h	$(8.2\pm0.9) \times 10^7$	$(2.6\pm0.2) \times 10^7$	$(1.9\pm0.8) \times 10^7$	$(1.2\pm0.3) \times 10^7$	$(1.8\pm0.5) \times 10^7$

Currently, these types of water-borne coatings are considered to be non-toxic, environmentally friendly, and safe. However, these results revealed that the water-borne coating material, which was not treated with any additional antimicrobial substances other than the in-can preservatives, surprisingly showed a noticeable influence on the bacterial growth (*Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*) (Table 2). This was caused by the biocidal preservatives in the coating materials, which penetrated the surroundings from

the sample and killed the bacteria. Samples without final in-can biocides were also tested. The same results obtained in the previous method were observed (Table 3). The reason for this was that the components themselves used for water-borne coating materials (*e.g*. binders) are treated with biocides for their protection. Although the biocides did not penetrate the surroundings from the sample, as in the case of the in-can preservatives (results in Table 1), bacteria inoculated on the sample surface were still killed by the antimicrobial substances used for the preservation of binders or coalescents.

Water-borne coating materials meeting the requirements for this type of product are used as the final treatment for wooden toys and wooden furniture for children because they are regarded as not harmful. These results confirmed that when in contact with a wet environment (*e.g.* saliva or sweat), the preservatives are released and could be dangerous, especially to children. Finally, it should be noted that the dry-film of the water-borne coating material tested according to the "wet test" JIS Z 2801 (2012) caused a 100% reduction in the tested bacteria (Table 2 and 3), while the dry-film of the polyurethane solvent-borne coating material caused no reduction in the bacteria (Table 4). Bacteria grew very well on the surface of the polyurethane solvent-borne coating material, which meant that no harmful antimicrobial substances were released from the samples.

Action against dry microbial contamination

The tests described above are all based on wet conditions in which bacteria are cultivated on the sample surface. Table 5 shows the results of a new testing method under dry conditions, which simulates real contamination by dust particles. The results of all three samples were comparable. Neither the water-borne nor the polyurethane solvent-borne coating materials significantly reduced the number of bacterial spores, which are very resistant. Taking into consideration the results of both testing methods, dry and wet, biocides used as in-can preservatives are effective only under wet conditions. These results indicated there is a serious problem for products that are in contact with liquids, such as toys for children, because biocides are allowed to diffuse from the coating materials to the mouth of a child *via* saliva or through sweat on the hands.

Table 5. Results of the Antibacterial Activity of the Water-Borne and Polyurethane Solvent-Borne Coating Materials Tested According to the Dry Bacterial Contamination Method

	Water-borne coating material	Water-borne coating material without in-can preservatives	Polyurethane solvent-borne coating material
CFU/sample 0 h	$(3.2\pm0.4) \times 10^2$	$(3.4\pm0.4) \times 10^2$	$(3.5\pm0.4) \times 10^2$
CFU/sample after 24 h	$(2.6\pm0.3) \times 10^2$	$(2.8\pm0.4) \times 10^2$	$(2.7\pm0.3) \times 10^2$

Evaluation of the Antifungal Activity

EN 15457 (2015)

The results of this test are expressed in Table 6. The polyurethane solvent-borne coating material did not exhibit any effect against fungal growth. The mix of tested fungi completely covered the polyurethane samples after 7 d (Fig. 5). In contrast, only 25% of the surface of the water-borne coating material samples was covered after this time (Fig.

4). Even after 3 weeks, the fungal growth on the dry-film of the water-borne coating material samples only covered 80% of the surface.

Table 6. Results of the Antifungal Activity of the Water-Borne and Polyurethane Solvent-Borne Coating Materials Tested According to EN 15457 (2015)

Sample	Fungal growth* after 7 d	Fungal growth* after 14 d	Fungal growth* after 21 d
Water-borne coating material	25%	70%	80%
Polyurethane solvent-borne coating material	100%	100%	100%
* mix of *Aspergillus niger*, *Cladosporium cladosporoides*, *Aureobasidium pullulans*, and *Penicillium funiculosum*			

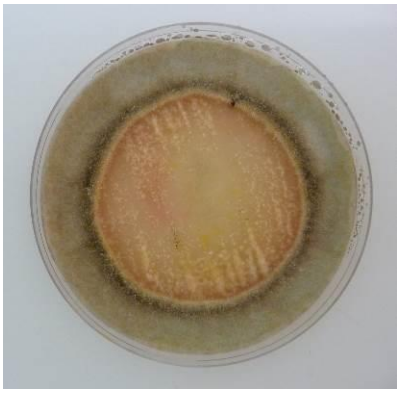

Fig. 4. Water-borne coating material after 7 d (EN 15457 2015)

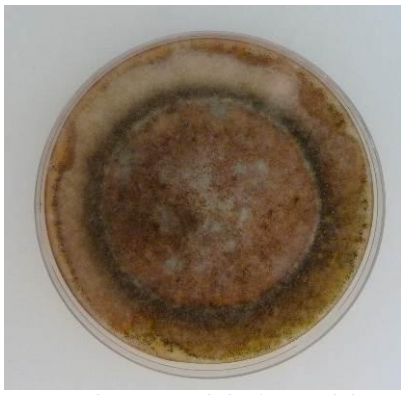

Fig. 5. Polyurethane solvent-borne coating material after 7 d (EN 15457 2015)

CONCLUSIONS

1. Water-borne coating materials with in-can biocidal preservatives have an antimicrobial effect, even after the formation of a dry-film. A 100% reduction in bacteria was observed for *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterococcus faecalis*, which were tested according to the method JIS Z 2801 (2012). Additionally, an antifungal effect against *Aspergillus niger*, *Penicillium funiculosum*, *Aureobasidium pullulans*,

and *Cladosporium cladosporoides* was observed. These results confirmed that there was a release of antimicrobial substances from the dry-film under wet conditions. This is a serious problem, especially in the case of wooden toys or products for children.

2. The polyurethane solvent-borne coating material had no antimicrobial effect against the bacteria and fungi after the formation of a dry-film.
3. A new testing method was presented for the evaluation of antimicrobial activity under dry conditions that simulated real contamination of furniture. An effect was not proven for both of the coating materials tested with this dry contamination method.

REFERENCES CITED

Athawale, V. D., and Nimbalkar, R. V. (2011). "Waterborne coatings based on renewable oil resources: An overview," *J. Am. Oil Chem. Soc.* 88(2), 159-185. DOI: 10.1007/s11746-010-1668-9.

Bravery, A. F. (1988). "Biodeterioration of paint - A state-of-the-art comment," in: *Biodeterioration*, D. R. Houghton, R. N. Smith, and H. O. W. Eggins (eds.), Springer Netherlands, Dordrecht, Netherlands, 466-485.

Buttner, M. P., Cruz-Perez, P., Stetzenbach, L. D., Garrett, P. J., and Luedtke, A. E. (2002). "Measurement of airborne fungal spores dispersal from three types of flooring materials," *Aerobiologia* 18(1), 1-11. DOI: 10.1023/A:1014977900352.

Köhler, T., Carlsen, A. T., and Tiedtke, G. (2011). "New solutions for in-can preservation in the making for Europe," in: *European Coating Conference*, Berlin, Germany.

Dancer, S. J. (2014). "Controlling hospital-acquired infection: Focus on the role of the environment and new technologies for decontamination," *Clin. Microbiol. Rev.* 27(4), 665-690. DOI: 10.1128/CMR.00020-14.

Davison, G. L. (2003). "Biocide review," in: *Additives in Water-borne Coatings*, G. Davison and B. Lane (eds.), Royal Society of Chemistry, Cambridge, UK.

Désor, D., Krieger, S., Apitz, G., and Kuropka, R. (1999). "Water-borne acrylic dispersions for industrial wood coatings," *Surface Coatings International Part B Coatings Transactions* 82(10), 488-496. DOI: 10.1007/BF02692644.

Directive 2009/48/EC (2009). "Directive 2009/48/EC of the European parliament and of the council of 18 June 2009 on the safety of toys," European Union, Brussels, Belgium.

Downey, A. (1995). "The use of biocides in paint preservations," in: *Handbook of Biocide and Preservative Use*, H. W. Rossmoore (ed.), Springer Science+Business Media, Dordrecht, Netherlands.

Ducel, G. F., Fabry, J., and Nicolle, L. (2002). *Prevention of Hospital-Acquired Infections: A Practical Guide, 2nd Edition* (WHO/CDS/CSR/EPH/2002.12), World Health Organization, Geneva, Switzerland.

ECHA. (2016). "Biocides supplier or user," (https://echa.europa.eu/support/getting-started/biocides), Accessed 6 November 2016.

EN 15457 (2015). "Paints and coating materials - Laboratory method for testing the efficacy of film preservatives in a coating against fungi," European Committee for Standardization, Brussels, Belgium.

Gillatt, J. (1998). "The use of biocides and fungicides in wood coatings and preservatives," *Surface Coating International* 81(7), 337-341. DOI: 10.1007/BF02700558.

JIS Z 2801 (2012). "Antimicrobial products – Test for antimicrobial activity and efficacy," Japanese Standards Association, Tokyo, Japan.

Khan, H. A., Ahmad, A., and Mehboob, R. (2015). "Nosocomial infections and their control strategies," *Asian Pacific Journal of Tropical Biomedicine* 5(7), 509–514. DOI: 10.1016/j.apjtb.2015.05.001.

Lindner, W. (2001). "New developments for in-can preservation of water-based paints and printing inks," *Surface Coatings International Part B: Coatings Transactions* 84(2), 141–146. DOI: 10.1007/BF02699776.

Makarewicz, E. N., Shiichuk, A. V., and Syrotyns'ka, I. D. (2011). "Quaternary ammonium salts as an antimicrobial additives to water-dispersible paints," *Russ. J. Appl. Chem.* 84(5), 888-891. DOI: 10.1134/S1070427211050259.

Regulation (EU) No 528/2012 (2012). "Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products," European Union, Brussels, Belgium.

Rijckaert, V., Stevens, M., Van Acker, J., de Meijer, M., and Militz, H. (2001). "Quantitative assessment of the penetration of water-borne and solvent-borne coatings in Scots pine sapwood," *European Journal of Wood and Wood Products* 59(4), 278-287. DOI: 10.1007/s001070100208.

Schleibinger, H. K., Keller, R., and Rüden, H. (2004). "Indoor air pollution by microorganisms and their metabolites," in: *The Handbook of Environmental Chemistry*, Springer Berlin Heidelberg, Berlin, Germany, 149-177.

Stetzenbach, L. D., Amman, H., Johanning, E., King, G., and Shaughnessy, R. J. (2004). "Microorganisms, mold and indoor air quality," (www.asm.org/ccLibraryFiles/FILENAME/000000001277/Iaq.pdf), Accessed 10 November 2016.

Wallström, E. H., and Hoffmann, L. (2001). "Environmental aspects of ISO and CEN standards," *Surface Coatings International Part B: Coating Transactions* 84(2), 113-119. DOI: 10.1007/BF02699772.

Article submitted: December 25, 2016; Peer review completed: February 18, 2017; Revised version received and accepted: March 15, 2017; Published: May 1, 2017.