Efficacy Requirements for Microbial Resistant Coatings

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From the beginning of human existence, mankind has shared the planet with microorganisms. We have evolved together and still interact constantly. The vast majority of such interaction is beneficial, even essential, to the survival of man. This interaction usually goes unnoticed. However, recent news stories about bird flu, SARS, and bioterrorism threats have reminded the public about the abundance of microorganisms around us and the relative ease that diseases and microbial-induced problems can be transmitted. Recent widely-publicized information about mold issues in poorly constructed homes and in the flooded homes along the U.S. Gulf Coast has served to bring mold and mildew issues to the forefront of many peoples' fears. Stories about moldy homes, poor indoor air quality, and dangerous black molds growing in air ducts contribute to anxieties about microbial-induced health risks in our daily environment. As a result manufacturers are experiencing an increased demand for surfaces in homes and public buildings to be treated in a manner that controls microbial growth on these surfaces, preferably over a prolonged period of time. Virtually every manufacturer of surfacing materials and coatings is determined to develop products to meet this need.

This article covers the application of biocides to prevent microbial growth in and on coating materials. This application falls under the "treated article exemption" of the U.S. Environmental Protection Agency (EPA). Under this exemption, treated articles, such as paints, are defined under Title 40 of the Code of Federal Regulations, Section 152.25(a), and are exempt from registration even though they contain biocides to prevent microbial deterioration of the coating. If any health claims are made or implied that result from using the treated coating, EPA regulations require the coating to be registered as a pesticide under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). This latter situation implies[1] high costs and is to be avoided by coating manufacturers.

This article describes experiments demonstrating which microbiological and physical properties are necessary for a biologically active substance to be successfully applied to coatings under practical (real world) conditions.

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MICROBIOLOGY OF PAINTS

There is no disagreement about the necessity to prevent microbial growth in paints in the wet-state by adding a proper bactericide. This has to be done or paint cannot be delivered to, or be used by, the end customer. As soon as the coating film dries, bacteria are no longer a threat and the applied bactericide ceases to function. For unprotected interior coatings in humid environments, fungi and yeast are most often found, while in exterior situations, algae, cyanobacteria (blue-green algae), as well as mold are common paint colonizers. Germination and growth of these ubiquitous organisms in and on dry coatings can be inhibited by a proper broad spectrum film preservative adapted to the intended use.

In nature, fungi grow on soil and plants and like to adhere to dust, soot, and pollen. The ultimate fungal source is rotting biomass in soil where a complex consortium of microorganisms is deteriorating the organic material produced by plants, animals, and other microbes. Especially in summer, air-borne fungal spore concentrations can be as high as 10^5 per cubic meter, and in the direct neighborhood of freshly harvested cereal grain fields, even much higher. Air-borne microbial spores and fragments are extremely mobile and can be found all over the world.

Fungi growing on interior coatings are seeded by spores from the outdoor air, which enter rooms through ventilation ducts, doors, windows, etc. In living rooms, even the soil in flower pots is a source of xerotolerant *Aspergillus* species. In bathrooms, where higher temperatures and air humidity are found, a shift to fungi with high urease activity is observed. *Cladosporium sphaerospermium* and *Aspergillus* species are commonly found in such situations.

While microbes are everywhere in the environment, they only have a chance to adhere and to germinate on interior coatings if the surface is humid. The different conditions found at interior walls dictate the growth of certain fungi species, like *Penicillium*, *Aspergillus*, *Fusarium*, *Mucor*, and *Stachybotrys*, which are found more frequently indoors than on exterior coatings. Yeasts are found only in rooms with high humidity like bathrooms. However, even in bathrooms, the available water on an interior coated surface is generally too low to allow bacteria to grow (see *Table* 1).

To prevent mold growth on interior coatings, the relative air humidity should not exceed 65%. Of course, this must be true for all parts of the room, even for the area behind the furniture and along the relatively cold exterior walls. In wet rooms where moisture is not easily controlled, fungal growth is often observed. In food processing environments yeast can grow on nutrient rich conditioned dirt layers. Fortunately, low dosages of an appropriate fungicide are sufficient to keep interior paints free from fungal growth.

In general, the moisture level does not affect bacteria growth on clean interior coating films. Growth and survival is possible in dirt layers which can retain high amounts of moisture. Such surface "conditioning" areas should be cleaned and disinfected on a regular basis. However, microbes living in conditioning dirt layers cannot be inhibited by non-migrating biocides in the paint film. Bacterial spores, e.g., *Bacillus anthracis* (Anthrax), are able to survive for long periods on surfaces and cannot be killed by permanent biocides incorporated in the substrate. However, they do not germinate on these surfaces except in very rare circumstances (see *Figure* 1).

Recently, antibacterial coatings claiming to offer a complementary approach to controlling bacterial levels in a wide number of institutional and private application areas have been entering the marketplace in increasing number. Predicated on the idea that bacteria can grow on surfaces in some circumstances, antimicro-

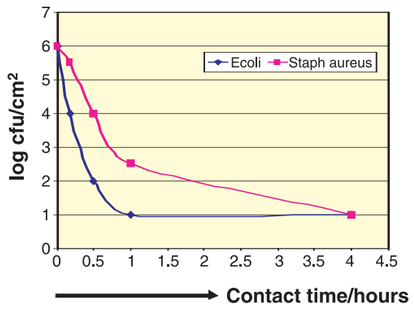
Table 1—Moisture Requirements of Microbes

Microorganism	Moisture min. aw	Moisture optimum aw
Bacteria	0.95	>0.99
Yeast	0.85	0.9 to 0.95
Hydrophilic mold (e.g., *Cladosporium spearospermum*, *Alternaria alternata*, *Stachybotris atra*)	0.75	0.85 to 0.9
Xerophilic mold (e.g., *Aspergillus versicolor*)	0.65	0.75 to 0.8

(a) Water activity—available water in a material (0 to 1).
Note: Water activity (humidity which is available for microorganisms in a substrate) is directly related to the air humidity.

Figure 1—Survival of bacteria on paint films.

- *Proliferating bacteria do not survive on a paint film.*
- *Spores (e.g., Bacillus anthracis (Anthrax)/Bacillus subtilis) are surviving for long periods of time, but do not grow.*



bial label claims have to be substantiated by experimental data and do not qualify for the treated article exemption. Such hypothetical situations are not the subject of this article.

MICROBIOLOGICAL TESTING OF ANTIMICROBIAL PAINTS

To cover both treated article-type and antibacterial coating-type applications, two different general types of testing methodology are required:

(1) Film preservation to prevent the growth of deleterious microbes on a coating as covered by the treated article exemption. The target organisms are, in general, different mold species. Recognized test methods applicable are ASTM D 3273 (mold chamber test) and ASTM D 5590 (agar diffusion test), which can be modified easily to special target organisms or application conditions. Such test methods are widely known and practiced in the industry.

Established test methods such as ASTM D 5590 and ASTM D 3273 can be complemented with artificial aging pretreatments like leaching to demonstrate long-term efficacy.

(2) Reduction of microbial cell numbers which are brought into tight contact with the coating that asks for an active kill effect of the coating. Target organisms are most often potentially pathogenic bacteria like *Staphylococcus aureus* or *Escherichia coli*, respectively. This application requires different test methods to demonstrate an antibacterial effect. Such application is not covered by the treated article exemption.

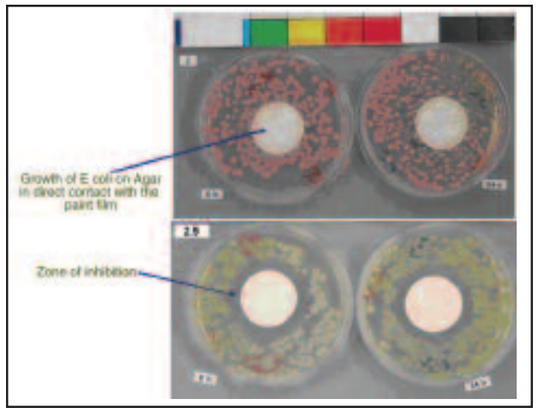
For the second situation, the traditional method is derived from the Swiss standard SNV 195120, which measures the inhibition of fast growing bacteria on an agar surface by bactericides in a paint film (see *Figure* 2). The method detects only migrating antibacterial substances. The test method is suited to simulate situations such as a surface spoilt with high loads of bacteria living in an organic matrix (e.g., sputum).

The Japanese standard JIS Z2801 (ISO DIS 22196) is a typical bacteria kill test requiring a log 2 reduction (99% kill) of an inoculum after 24 hr contact time relative to an unequipped surface (*Figure* 3).

Inherent to both types of tests is the necessity to keep the bacteria artificially moist to ensure survival of the inoculum on the blank paint films not containing a biocide. Criticism of the test procedures centers on these artificial conditions, lack of soiling and aging of the surface, the arbitrarily chosen contact time of 24 hr, and the test temperature of 35°C (95°F). Regulatory bodies will question the practical benefits of antibacterial treatments and a battery of more relevant in-use tests are to be developed. For example, what is the benefit of having an antimicrobial door knob with a bactericide which can reduce the bacterial count on the surface at 35°C by 2 logs within 24 hr if the next person is entering only one minute after the inoculation?

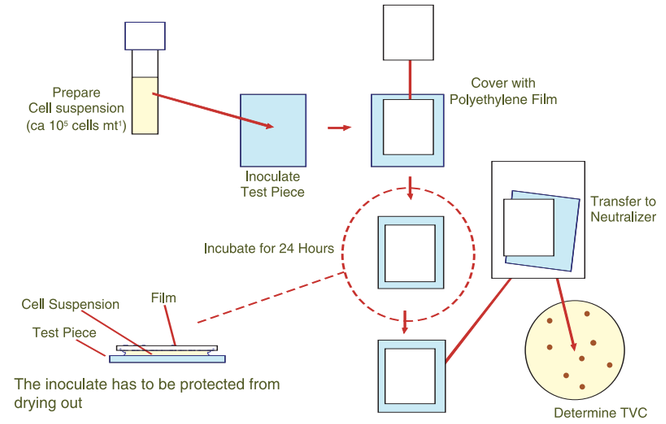
Regardless of the controversy regarding antibacterial coatings, a real existing need is the prevention of fungal growth on surfaces in humid interior environments, e.g., laundries, bathrooms, and kitchens. Growth of fungi on such surfaces directly follows the moisture patterns exhibited at the surface. In such cases a film preservative (fungicide) is required. A film fungicide has to provide a long-term inhibition of fungal spore germination and growth of fungal hyphe.

Figure 2—Growth inhibition test for antibacterial paints according to Swiss Standard SNV 195120. The *Escherichia coli* colonies are visualized by a redox-indicator added to the agar.



The fungicide has to be effective even in the presence of attached moist dirt (soiling). To achieve this function, the biocidal active substances must have a certain, but limited, mobility in the paint film as well as extremely high activity. As an example, modern film preservatives, like Polyphase 678, are formulated to have no detrimental effect on the desired paint properties, to not contribute to the VOC level, and to be effective both for clean and soiled surfaces.

Figure 3—JIS Z2801 bacteria kill test. Quantitative count of survival bacteria after 34 hr contact time. The bacteria have to be kept wet by a cover slip to avoid dry out.



MOBILITY OF COATING FILM BIOCIDES

To understand how a microbicide can protect a paint film and the adhered dirt layer against fungal growth, both biological and physical parameters have to be considered.

A typical paint film is a complicated multiphase system with pigments and extenders embedded in an organic binder matrix containing variable amounts of paint additives which have a function in the wet paint (for example, rheology modifiers, coalescent solvents, and in-can preservatives to name but a few, as well as water). In a paint film with low PVC, the pigment particles are completely enveloped in the binder matrix and the surface of the fresh coating consists mainly of the polymer material. As mentioned earlier, fungi which come into contact with the paint film have to either adhere directly to the surface or on a dirt layer coating the surface.

Next, every material exposed to environmental conditions tends towards a humidity equilibrium with the environment which often is favorable for fungal growth. To inhibit attachment of fungal cells and subsequent germination and growth, the coating environment has to have a higher level of the fungicide than the minimal inhibition concentration (MIC). However, this is not the concentration of the fungicide added to the paint, but rather the *available concentration* in the humid layer within which the fungal cell is situated. In a coating, the available concentration of the fungicide is the phase transition equilibrium of the fungicide between the organic binder material and the exterior humid environment where the fungal cell exists (*Figure* 4).

Such a model can be used to clarify and dispel some myths that exist in the industry, some for years, and some of more recent vintage:

1. Availability at the surface does not necessarily mean fast depletion from the paint film.

2. Biocidal actives with high solubility in coalescent solvents like 3-Iodpropinyl-N-n-butylcarbamate (IPBC) do not enrich at the surface with the evaporating coalescent as some have stated,[2] nor does the coalescent enrich at the paint surface.

3. Biocidal actives like Zinc Pyrithione (ZPT) or 2-Methoxycarbonylamino benzimidazole (BCM–Carbendazim), which do not easily dissolve in the coating polymer matrix, must have an extremely high activity against fungi to be effective at the location where the fungi are thriving.

Figure 4—Available biocide concentration at the surface of a paint film strives towards an equilibrium depending on the local concentration in the paint binder.

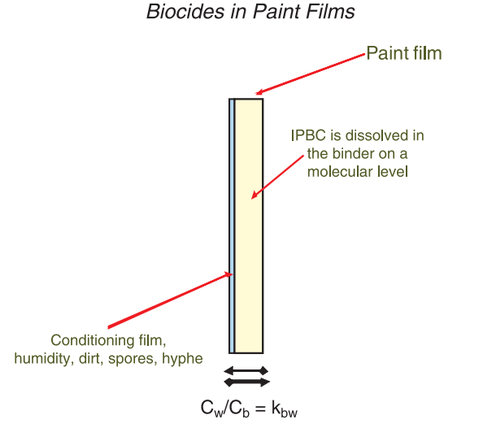
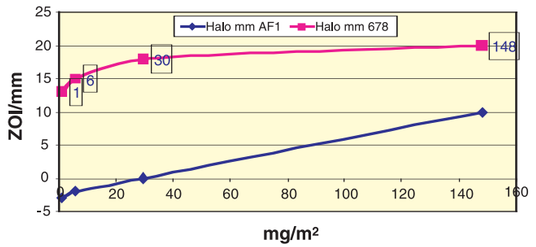


Figure 5—Activity of BCM and IPBC in a high PVC coating. The zone of inhibition (ZOI mm) is a measurement of activity against the test fungus *Aureobasidium pullulans*.



1 mg per m² of an available highly active film fungicide is enough to inhibit the growth of *Aureobasidium pullulans* not only directly on the coating but also in its vicinity.

Figure 6—Multilayer test design to demonstrate the mobility of active substances over the cross section of a paint film. Pure acrylic paint: Troy Recipe #8, PVC = 40.

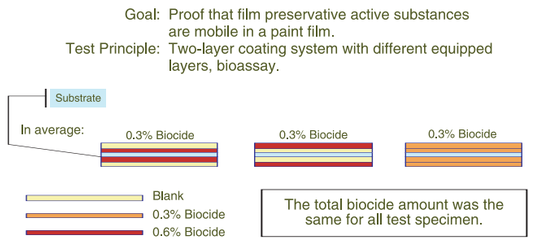
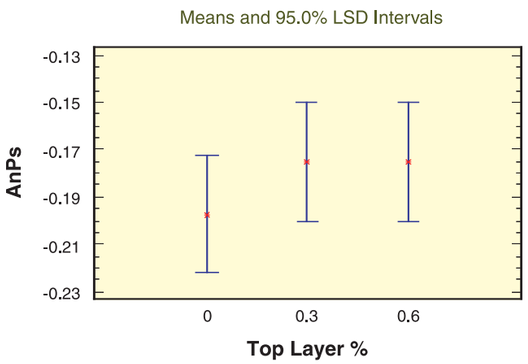


Figure 7—Mobility of IPBC in a paint film. Note: ANOVA of the zone of inhibition size (ZOI in mm/20) shows that the result is independent from the structure of the layered test specimen, the ZOI is the same for the test specimen with no biocide, and 0.3% or 0.6% of the biocide in the top layer. This proves the establishment of an equilibrium concentration over the entire paint cross section. AnPs: Test *Fungi Aspergillus niger* and *Penicillium* species.



The halo was independent from the layer structure.

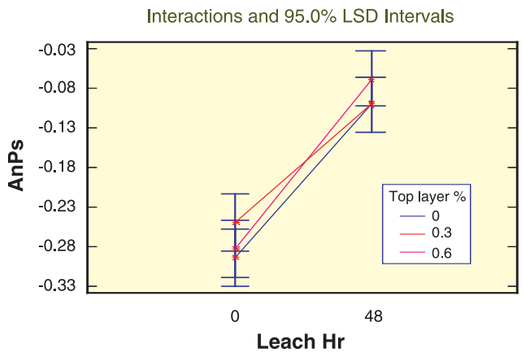
To illustrate a recent example of one of the myths, an experiment was published[2] proposing to demonstrate the enrichment of IPBC at the surface of an undercritical paint film (PVC=40) using ESCA (electron spectroscopy for chemical analysis and Auger spectroscopy). The published elemental composition of the paint film surface demonstrates nicely the mainly organic nature of the surface as expected. The reported average iodine concentration was 1%, which is not very far from expectation assuming the unpublished paint recipe has 40% of a polymer emulsion and the pigments and fillers do not adsorb IPBC. Under these assumptions, a local concentration of 2% IPBC in the cured binder can be calculated.

The problem is that the ESCA method, with its limited penetrability, is absolutely unsuited to judge on the distribution of paint additives over the cross section of the paint film. Only data points up to a depth of 0.007 micrometer and only at a very few spots were reported, which is by far not representative of the entire cross section. To put the issue into perspective, it might be remembered that the grain size of pigments and fillers are in the range of 2 to 40 micrometers in a paint film. Conclusions that there is an enrichment of IPBC at the paint surface are very far fetched and not supported by the experimental data.

Because of such confusion about the chemical motility of various fungicides, experiments were undertaken to demonstrate the necessary activity of the film fungicides IPBC and BCM to be judged effective, as well as the actual distribution of such fungicides over the cross section of the paint film.

To test the activity of IPBC and BCM, a ladder series of the active substances were incorporated into a low

Figure 8—Leaching effect per sandwich type. Note: The different structure of the paint film does not influence the leaching velocity from the total paint film.



The efficacy of IPBC is independent from the method the film was built up.

binder-containing paint. Draw-downs were made on Whatman No. 1 filter paper, dried, and leached in tap water for 24 hr to remove soluble ingredients which might have inhibitory effects. After drying, the paint films were exposed on malt agar to the attack of *Aureobasidium pullulans*, a typical and common black-colored paint colonizer. After 14 days incubation, the test specimens were rated according to ASTM D 5590 and the growth free zones of inhibition (zoi in mm) were measured in the vicinity of the test specimen.

The results are summarized in *Figure* 5 and expressed as amount of active substance per area unit. An amount of only 1 mg BCM per m² of coated surface is enough to generate a large zone of inhibition of 13 mm. These data demonstrate the extremely high activity of modern film fungicides. Further, this high activity demonstrates how relatively immobile preservatives can have any effect for soiled coating surfaces.

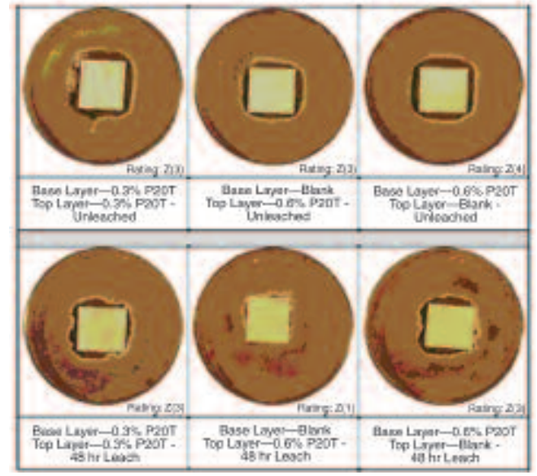
Why is such high activity necessary? Because only low levels of the active substances are available in the humidity layers where the fungi are growing.

The next experiment was designed to demonstrate the mobility of the active substances over the cross section of paint films and the availability of the active substance portions in all sections of the coating. Diffusion is the main transport mechanism over the cross section of the paint film. This mechanism allows the total utilization of the applied biocide load in a coating and describes the long lasting action of a film protection with Polyphase 678, for example.

Troy Test Paint Recipe #8, based on a pure acrylic binder (AC264) with a PVC of approximately 40, was produced. The paint was divided into three parts: to one part 0.6% of Polyphase P20T (20% IPBC) was added, to the second part 0.3% Polyphase P20T was added, and the third part was left as film preservative free. According to the scheme outlined in *Figure* 6, the paint samples were drawn down on both sides of a Whatman No. 1 filter paper, here called the base layer. After drying at ambient temperature, a second layer was applied on top of the base layer (called the top layer in the illustration).

The first paint film test specimen had a base layer with no biocide and 0.6% in the top layer. The second specimen had 0.6% of the biocide in the base layer and no biocide in the top layer, and the third paint film test specimen had 0.3% of the biocide in both layers. With that arrangement, every test specimen had the same total amount of film preservative, equivalent to 0.3%. If the IPBC were not mobile in the test paint film, or the active substance enriched at the surface with the evaporating coalescent, the three differently structured test specimens should show different fungal inhibition patterns in ASTM D 5590. Specifically, the

Figure 9—IPBC (Polyphase P20T)—Equilibrium time three weeks at 4°C. Note: Equal fungicidal effects were found with differently structured multilayered paint films, suggesting the equilibrium of the IPBC concentration via the cross section of the paint film within three weeks at 4°C.



second test system with no biocide in the top layer should be overgrown by the test fungi, while the specimen with 0.6% in the top coat should show the largest zone of inhibition.

On the other hand, if the IPBC distributes evenly in the polymeric binder by diffusion over the total cross section of the paint film, the three differently layered test specimen should provide the same results.

All tests were run in triplicate, and other parameters like the equilibrium time and leaching regimes were investigated as well (data not shown). The data results were subjected to analysis of variances (ANOVA) and it was found that the ASTM D 5590 results were statistically identical regardless of the original structure of the layered test specimen. The IPBC must have been mobile within the cross section of the paint film and must have equilibrated after the paint film layers were dried (*Figure* 7). Further support of this conclusion was gained from the leaching experiments (*Figure* 8). No differences in the leaching behavior were seen when comparing the three different multilayered paint films. The results suggest that depleted IPBC at the paint surface is quickly replenished from the bulk paint film.

Similar results were obtained with Polyphase 678, demonstrating the excellent availability of BCM and IPBC at the paint surface (*Figure* 9). Further active film preservative substances and different coating substrates were investigated. The results will be published elsewhere.

CONCLUSION

By simple microbiological experiments according to ASTM D 5590 performed on multilayered paint film species and with different biocide concentration gradients over the cross section, it can be demonstrated that:

- IPBC distributes homogenously in the paint binder
- No enrichment of IPBC at the surface of the paint film while drying is occurring
- The mobility of IPBC in the binder polymer is high and depleted IPBC is replenished from the bulk
- The migration of IPBC over the paint film cross section is following the concentration gradient

SUMMARY

Adding a permanent microbiocide to a coating material can have two different intentions: an active kill effect to microbes coming into contact with the coating, and the preservation against paint disfiguring or deteriorating organisms. In the second case the microbicide does nothing but protect the coating against colonization, while in the first case the coating has to have active biocidal properties. These two intended biocide uses are treated differently by regulatory bodies, and require different test methods to demonstrate efficacy.

The antimicrobials for paints must meet the regulatory standards in the individual markets and must demonstrate experimentally a benefit to the end user. Paint films in interior environments are subject of colonization by fungi and yeasts, while bacteria in general do not find the conditions hospitable for growth.

Film preserving biocides for interior coatings must therefore be effective with a broad based activity towards fungi and should meet the other requirements for environmentally acceptable paints (especially zero VOC). The antimicrobial activity must come together with favorable physical properties to improve the resistance of the coating. For a modern zero-VOC paint film preservative based on IPBC and BCM, efficacy and active substance mobility data support the favorable real-world experience. CT

References

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(2) Kappock, P., "Fungicide Concentration Gradient: A Formulation Variable," *Modern Paint Coat.*, p. 25 (1997).