TROY CORPORATION

MICROBIOLOGY LABORATORY REPORT

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ANTI-MICROBIAL EQUIPMENT OF EPOXYDEN-EPOXY BASED FOR HYGIENIC FLOOR PAINT
TEST OF THE FUNGAL RESISTANCE ACCORDING TO TROY AGAR DIFFUSION TEST METHOD
TEST OF THE ANTIBACTERIAL SURFACE PROPERTIES ACCORDING TO ISO22196 (JIS Z2801)

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CONFIDENTIAL

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PROJECT BACKGROUND AND OBJECTIVE:

A Silver ion based antimicrobial from S&M in epoxy paint is used as antimicrobial floor paint for hospitalstes alternative organic antimicrobials in the two component epoxy floor coating system
This report is for the antifungal effect of the laddered epoxy coating as well as the anti-bacterial effect of the surface tested according to ISO22196 (JIS Z2801)

SAMPLE IDENTIFICATION:

TABLE 1: SAMPLE LABELS

SPNO	SPECIMEN	NOTES
01	Epoxyden mid/top coat	as received: BLANK - Epoxy resin (2 component epoxy top layer); about 1000 g.
01.01	Epoxyden mid/top coat + 0.6% EX2200	Antibacterial added to the epoxy
01.02	Epoxyden mid/top coat + 0.3% Polyphase FX40	Fungicide added to the epoxy
01.03	Epoxyden mid/top coat + 0.3% EX2200 + 0.3% Polyphase FX40	Antimicrobial combination added to the epoxy
01.04	Epoxyden mid/top coat + 0.3% Troysan S97	Antimicrobial added to the epoxy
02	Epoxy Hardener	as received: BLANK - Epoxy Hardener; about 500 g.
03	50 parts 01+ 25 parts 02	Blank - mix apply and let dry
04	50 parts 01.01 + 25 parts 02	EX2200 (immobilized BIT)
05	50 parts 01.02 + 25 parts 02	Polyphase FX40
06	50 parts 01.03 + 25 parts 02	EX2200 (immobilized BIT) + Polyphase FX40
07	50 parts 01.04 + 25 parts 02	Troysan S97
08	Positive Control	Architectural paint which was demsonstrated to be suscpetible to bacteria (added by TTCA as control of the test system)

- Samples were received on November 15 2011.
- SPNO is the laboratory-assigned identifier.
- Sub-samples were prepared with the addition of the microbicides as on Table 1.
- Test Specimen (03 to 07) were produced at TTCA by mixing epoxy and hardener at the described ratio
- Additional sample description is on Table 2.

SUMMARY AND CONCLUSION:

Antibacterial Effect JIS Z2801

The blank (SPNO 01) supported growth the test bacteria after 48-hour leaching but not after 1-hour leaching.

Only the addition of 0.3% Troysan S97 (SPNO 07) showed an anti-bacterial effect against *Escherichia coli.*

All other additions did not prove anti-bacterial effect after 48-hour leaching in this study.

None of the equipments passed the criteria of an at least log 2 reduction (99% kill) of the Japanese Standard JIS Z2801.
Probably the active substances are too well immobilized in the expoxy system

Resistance to Fungal Growth

The blank (SPNO 01 Epoxyden mid/top coat + SPNO 02 Epoxy Hardener) was not heavily grown but tends to be more susceptible with longer leaching.

Polyphase FX40 provided protection against fungal growth.
Troysan S97 confirmed consistent resistance against the combined *Aspergillus niger* + *Penicillium funiculosum.*

Test results are presented on Results Tables and attached photos.

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All information and recommendations furnished are for guidance and are without guarantee.
All preservatives should be field tested prior to use in actual manufacturing

RESULTS TABLES

TABLE 2: SAMPLE DESCRIPTION

SPNO	SPECIMEN	COLOR	STATE
01	Epoxyden mid/top coat	Light grey	paste
01.01	Epoxyden mid/top coat + 0.6% EX2200	Light grey	paste
01.02	Epoxyden mid/top coat + 0.3% Polyphase FX40	Light grey	paste
01.03	Epoxyden mid/top coat + 0.3% EX2200 + 0.3% Polyphase FX40	Light grey	paste
01.04	Epoxyden mid/top coat + 0.3% Troysan S97	Light grey	paste
02	Epoxy Hardener	Light yellow	liquid

Neither Discoloration or viscosity change was observed on any of the Epoxy components equipped with antimicrobials

Neither Discoloration and cracking was observed on any of the produced coating films

TABLE 3: ANTI-BACTERIAL FILM PRESERVATION TEST (JIS Z2801-TR)
The paint films were leached 1h prior testing

SPNO	Antimicrobial	CFU recovered *Ec* L 1h	Log reduction *Ec*	CFU recovered *Sa* L 1h	Log reduction *Sa*
03	**Blank - mix apply and let dry**	0	basis	0	basis
08	Positive control	10 EXP 5	control	10 EXP 5	control

Test Organisms: *Ec=Escherichia coli*; ***Sa***= *Staphylococcus aureus*

Leaching treatment: L 1h = 1 hour in water

CFU: number of colony forming units (bacteria cells) recovered after 24h incubation (for the efficacy calculation the logarithm of the cfu is formed (example EXP 4 means 10 0000 cfu, which logarithm log (cfu) is 4)

Basis: the unfortified sample, which is the basis of log reduction calculation of the following fortified samples

NS: not suitable, as the basis (the unfortified test film) did not support bacterial growth

Result Interpretation

As there could not be established a survival of the test bacteria on the non-fortified sample the effect of antimicrobials in this coating could not be assessed after 1h leaching. The coating itself demonstrated the antibacterial effect compared to a non-fortified control sample

TABLE 4: ANTI-BACTERIAL FILM PRESERVATION TEST (JIS Z2801-TR)

Leached 48h

SPNO	TESTMETHOD	CFU recovered *Ec* L 48h	Log reduction *Ec*	CFU recovered *Sa* L 48h	Log reduction *Sa*
03	**50 parts 01(no fungicide) + 25 parts 02 (Hardener)**	10 EXP 5	basis	10 EXP 5	basis
04	**50 parts 01.01 (EX2200) + 25 parts 02**	10 EXP 5	<2	10 EXP 5	<2
05	**50 parts 01.02 (Polyphase FX40)+ 25 parts 02**	10 EXP 5	<2	10 EXP 5	<2
06	**50 parts 01.03 (EX2200 + 0.3% Polyphase FX40) + 25 parts 02**	10 EXP 5	<2	10 EXP 4	<2
07	**50 parts 01.04 (Troysan S97)+ 25 parts 02**	0	>2	10 EXP 5	<2
08	Positive control	10 EXP 5	control	0	control

Test Organisms: *Ec=Escherichia coli*; ***Sa***= *Staphylococcus aureus*

Leaching treatment: L 48h = 48 hours in water

CFU: number of colony forming units (bacteria cells) recovered after 24h incubation (for the efficacy calculation the logarithm of the cfu is formed (example EXP 4 means 10 0000 cfu, which logarithm log (cfu) is 4)

Basis: the unfortified sample, which is the basis of log reduction calculation of the following fortified samples

NS: not suitable, as the basis (the unfortified test film) did not support bacterial growth

Result Interpretation (Log reduction):
>2 = passed
<2 = failed

None of the equipments passed the criteria of an at least log 2 reduction (99% kill) of the Japanese Standard JIS Z2801.
Probably the active substances are too well immobilized in the expoxy system

TABLE4: ANTI-FUNGAL TESTS (Troy Agar Diffusion Method) AD TR

SPNO	SPECIMEN	*Aa* L 1h	*An+ Pf* L 1h	*Aa* L 48h	*An+ Pf* L 48h
03	50 parts 01(no fungicide) + 25 parts 02 (Hardener)	1	1	2	2
04	50 parts 01.01 (EX2200) + 25 parts 02	2	2	3	2
05	50 parts 01.02 (Polyphase FX40)+ 25 parts 02	1	1	1	1
06	50 parts 01.03 (EX2200 + 0.3% Polyphase FX40) + 25 parts 02	1	1	1	1
07	50 parts 01.04 (Troysan S97)+ 25 parts 02	1	1	2	1

Leaching Treatment: **L 1h**= 1 hour in water
L 48h= 48 hours in water

Test organisms: *Aa* = ***Alternaria alternata***
An+ Pf = ***Aspergillus niger* + *Penicillium funiculosum***

GROWTH RATING SYSTEM:

0Z: No growth on the specimen, zone of inhibition may be present
0: No growth on the specimen surface
1: less than 10% of the specimen surface covered
2: less than 30% of the specimen surface covered
3: less than 60% of the specimen surface covered
4: more than 60% of the specimen surface covered or specimen surface totally overgrown

MATERIALS AND TEST PROCEDURES:

The samples were tested for **Anti- fungal Preservative Properties** in accordance with the TROY test methods.

Anti-fungal Properties:

The preservatives were added to Epoxyden mid/top coat then mixed with Epoxy Hardener as on Table 1.
From the prepared mixtures of Epoxyden mid/top coat and Epoxy Hardener, a layer of sample (300µm) was applied onto filter paper then air-dried for 7 days.
Leaching treatments were done as specified on Result Tables.
The prepared samples were then placed on the seeded Malt Extract Agar and incubated for 2 weeks at 28°C. The fungal inocula used *were Alternaria alternata;* and *Aspergillus niger* + *Penicillium funiculosum.*

All prepared samples were sterilized by γ-radiation at a level of ±35 kGy prior to the test with microorganisms.

All organisms are held on culture in the Microbiology Laboratory.

Annex 1: Photos of Anti-fungal Tests