Performance, Safety and Compatibility Microcyn™ technology

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Introduction

This document provides an overview of the performance, safety and compatibility study results, related to the Microcyn™ technology and is intended to list the characteristics of the Microcyn™ technology in general. This technology is the basis for individual product applications, like Dermacyn™ Wound Care, Oculus Microcyn$_{60}$ Disinfectant, etc.

Disclaimer:
The information below shall not be interpreted as the claims for individual product applications. Regulations are specific per product application and individual regulatory approval (e.g. CE certification per the Medical Devices Directive 93/42/EEC) is required. Please refer to the Instructions For Use for the applicable product for specific product claims.

Performance

Bactericidal (carrier test)

The bactericidal characteristics of Microcyn™ were tested using the AOAC Use-Dilution method, a carrier type test, as required per the United States EPA DIS/TSS-01 guidelines. The tested organisms were *Pseudomonas aeruginosa* (ATCC #15442), *Staphylococcus aureus* (ATCC #6538) and *Salmonella choleraesuis* (ATCC #10708). Per organism 60 samples were used for testing the bactericidal properties of Microcyn™. Each sample consisted of a stainless steel cylinder with a film of the specific bacteria dried onto it. The cylinders were exposed for 10 minutes to Microcyn™ and afterwards transferred to vessels containing a subculture medium. After incubation of 48 hours at 35°C ± 2°C, the samples were observed for surviving bacteria.
Conclusion: *for each organism a bacterial load reduction of at least 5.4 x 10^4 was achieved, while one or none out of 60 carriers showed positive growth thus complying with the EPA requirements.* [ATS labs., United States of America]

Bactericidal (suspension test)

The bactericidal properties of Microcyn™ were tested in accordance with the BS EN 13727:1999 'draft' standard, a suspension type test. Per organism, bacterial suspensions with a concentration between 1.5 x 10^8 and 5.0 x 10^8 cells / mL were prepared for *Pseudomonas aeruginosa* (ATCC #15442), *Staphylococcus aureus* (ATCC #6538) and *Enterococcus hirae* (ATCC #10541). From each of the bacterial suspensions 1 mL was added to 8 mL of Microcyn™ together with 1 mL of bovine albumine (0.03 g/L) as an interfering substance. The test mixture was kept at 20°C for 15 minutes and then plated out on tryptic soy agar plates. After 48 hours of incubation at 36°C ± 1°C, the plates were observed for growth.
Conclusion: *for all three tested organisms a reduction of the bacterial load of at least 10^5 was achieved, within a contact time of 15 minutes with Microcyn™ in five replicas performed in duplex.* [Micromed Laboratories Inc., United States of America]

Bactericidal: resistant strains (MRSA)

In this test, the bactericidal characteristics of Microcyn™ against Methicillin resistant *Staphylococcus aureus* (ATCC #33592; MRSA) were tested, per the DIS/TSS-05 guidelines. Using the AOAC Use-Dilution method, 20 penicylinders with a bacterial film dried on them were immersed for 10 minutes in Microcyn™. Following exposure the cylinders were transferred to neutral culture medium and incubated for 48 hours at 35 - 37°C. After incubation the samples were observed for surviving bacteria.
Conclusion: *under the conditions of this investigation, all 20 samples showed no growth, which implies a bacterial load reduction of 8.8 x 10^5, and thus proving compliance with the EPA requirements.* [ATS labs., United States of America]

Bactericidal: resistant strains (VRE)

In this test, the bactericidal characteristics of Microcyn™ against Vancomycin resistant *Enterococcus faecalis* (ATCC #51299;VRE) were tested, per the DIS/TSS-05 guidelines. Using the AOAC Use-Dilution method, 20 penicylinders with a bacterial film dried on them were immersed for 15 minutes in Microcyn™. Following exposure the cylinders were transferred to neutral culture medium and incubated for 48 hours at 35 - 37°C. After incubation the samples were observed for surviving bacteria.
Conclusion: *under the conditions of this investigation, all 20 samples showed no growth, proving compliance with the EPA requirements. In all samples a reduction of the bacterial load of at least 1.45 x 10^6 was achieved.* [ATS labs., United States of America]

Tuberculocidal (Mycobacterium)

In this test the bactericidal properties of Microcyn™ were tested against *Mycobacterium bovis* (OT #105401) in accordance with DIS/TSS-06 EPA guidelines for tuberculocidal testing. Bacterial suspensions were prepared with a 5% organic load (fetal bovine serum). A single tube was inoculated with the bacterial suspension followed by exposure to Microcyn™ for 5 minutes.
Conclusion: *during incubation for 20 days at 35°C ± 2°C a bacterial load reduction of at least 2.5 x 10^6 was achieved and Microcyn™ was found to comply with the tuberculocidal requirements of this investigation.* [ATS labs., United States of America]

Virucidal

Microcyn™ was tested to determine the virucidal characteristics against the Human Immunodeficiency Virus type I (HIV-I), Strain HTLV-IIIB, in accordance with the United States EPA DIS/TSS-07 guidelines. The virus was applied as a dried film on sterile, glass Petri dishes, followed by exposure to Microcyn™ for 10 minutes. Subsequently the virus was separated from the test substance by gel filtration and titered by serial dilutions for infectivity assay.
Conclusion: *per dilution groups (7 groups: 10^1 through 10^7), 8 samples were observed and in no sample infective activity was detected. The reduction in viral titer was at least 5.6 x 10^3 for all samples. Under the conditions of this investigation, Microcyn™ demonstrated complete inactivation of HIV-I following a 10 minute exposure time, in accordance with virucidal requirements as defined by the EPA.* [ATS labs., United States of America]

Fungicidal

Microcyn™ was tested to determine the fungicidal characteristics against the fungus *Trichophyton mentagrophytes* (ATCC #9533). The test was conducted in accordance with the DIS/TSS-06 EPA guidelines, using the AOAC Use-Dilution method. Fungal cells were applied as a dried film on stainless steel carriers and were exposed for 10 minutes to Microcyn™, containing a 5 % fetal bovine serum organic load. Following exposure the carriers were transferred to neutralizing subculture medium and assayed for survivors upon incubation.
Conclusion: *under the conditions of this experiment, Microcyn™ was fungicidal against Trichophyton mentagrophytes following a ten minute exposure at 20°C. In all samples a bacterial load reduction was achieved of at least 2.5 x 10^6.* [ATS labs., United States of America]

Sporicidal

Microcyn™ was tested to determine sporicidal charasteristics against spores of *Bacillus atrophaeus* (ATCC #6633). The test was conducted in accordance with the BS EN 14347:2002 'draft' standard. A spore solution with a concentration of 10^8 - 10^9 spores / mL was prepared from a purchased spore suspension. Test samples contained 1 mL of the spore solution, 1 mL water and 8 mL Microcyn™. The spore solution was exposed for 15 minutes to Microcyn™, after exposure the samples were plated and incubated for four days at 36 ± 1°C.
Conclusion: *under the conditions of this experiment, Microcyn™ was sporicidal against Bacillus subtilis spores. The reduction in spores was at least 3.2 x 10^6, thus proving compliance with the requirements of the applied test method.* [ATS labs., United States of America]

Safety / Biocompatibility

Genotoxicity

Microcyn™ was evaluated for its genotoxic potential according the OECD guideline #474 Mammalian Erythrocyte Micronucleus Test, per the ISO 10993-3:2003 standard. The test substance was intraperitoneal injected into a test population of 5 male and 5 female mice. The applied dose was 12.5 mL Microcyn™ / kg body weight for 2 consecutive days. After injection the mice were observed for general health and adverse reactions. On the third day the mice were terminated and the ratio Polychromatic erythrocytes/Normochromatic erythrocytes was determined. In addition the polychromatic erythrocytes were examined for the incidence of micronucleation.

Conclusion: *clinical observations revealed no signs of toxicity during the study. Microscopic examination of bone marrow smears of the subjected animals showed absence of toxicity in the treated animals comparable to the result found in the negative test, where a 0.9 % NaCl solution was used. The same comparison was made for the Polychromatic Erythrocytes bearing micronuclei. In both the subjected animals and the animals from the negative test this number did not increase, showing the absence of mutagenic effects. Based on this study, Microcyn™ is concluded to cause no genotoxic effects.* [Biomatech S.A.S., France]

Cytotoxicity

This test was executed in accordance with ISO 10993-5:1999 standard to determine the potential of Microcyn™ to cause cytotoxicity. A filter disc with 0.1 mL of Microcyn™ was placed onto an agarose surface, directly overlaying a monolayer of mouse fibroblast cells (L-929). The prepared samples were observed for cytotoxic damage after 24 hours of incubation at 37°C in the presence of 5% CO_2. Observations were compared to positive and negative control samples.

Conclusion: *Microcyn™ containing samples did not reveal any evidence of cell lysis or toxicity, while positive and negative control performed as anticipated. Based on this study Microcyn™ was concluded not to generate cytotoxic effects.* [Namsa, United States of America]

Dermal Sensitization

A skin sensitization study was conducted on 15 male and 15 female short-haired albino guinea pigs to determine if Microcyn™ produces a sensitizing reaction. The test is performed in accordance with the US EPA OPPTS 870.2600 guidelines and is required for compliance with the ISO 10993-10:2002 standard. In a naive control group 5 animals from each sex were incorporated. These animals remained untreated for five weeks, hereafter they were given a single dose of 0.4 mL Microcyn™. The remaining animals were treated once every week for three weeks with 0.4 mL Microcyn™, rested for 2 weeks and in the fifth week of the experiment once again treated with 0.4 mL Microcyn™.

Conclusion: *no skin irritation was detected in the animals from the naive control group. Neither any irritation was detected in the remaining group. Because of the absence of any skin response Microcyn™ was concluded not to be a skin sensitizer.* [Stillmeadow, Inc., United States of America]

Skin Irritation

A test was executed to comply with the ISO 10993-10:2002 standard to determine the skin irritation potential of Microcyn™. In this test, on three New Zealand White rabbits a dose of 0.5 mL Microcyn™ was applied to the assigned test sites on the animal. The test substance was dosed on gauze and attached to the test sites to generate 4 hours of skin contact. The treated skin was observed for 72 hours at regular time intervals and compared to an untreated skin site on the same animal. The animals were observed for formation of edema, erythrema and/or eschar formation.

Conclusion: *all animals were encountered healthy during the complete study. No skin irritation was detected throughout the total study period. Based on this study, the skin irritation of Microcyn™ is concluded to be negligible.* [Northview Pacific Laboratories Inc., United States of America]

Ocular irritation

A test was executed to comply with the ISO 10993-10:2002 standard to determine the ocular irritation potential of Microcyn™. In this test, on three New Zealand White rabbits a dose of 0.1 mL Microcyn™ is instilled into the right eye of each animal. The left eye was left untreated and served as a control. After application of the test substance eyes were examined at regular time intervals for evidence of irritation.

Conclusion: *all animals were encountered healthy during the complete study. No ocular irritation was detected throughout the total study period. Based on this study, it is concluded that Microcyn™ does not cause ocular irritation.* [Northview Pacific Laboratories Inc., United States of America]

Acute Oral toxicity

Microcyn™ was evaluated for its acute oral toxicity potential when administered as a gavage dose at a level of 5000 mg / kg body weight to a test population existing of 3 female albino rats. The test was executed in accordance with US EPA OPPTS 870.1100 regulations, to comply with the ISO 10993-11:1996 standard. The subjected animals received 4.98 mL Microcyn™ / kg body weight and were observed for 14 days.

Conclusion: *no mortality or clinical / behavioral signs of toxicity were detected. The gross necropsy conducted at the end of the study revealed no observable abnormalities. Based on this study, Microcyn™ is concluded not to cause oral toxicity.* [Stillmeadow, Inc., United States of America]

Acute Dermal Toxicity

A test was executed in accordance with US EPA OPPTS 870.1200 regulations to determine acute dermal toxicity and relative skin irritancy of Microcyn™, as required for compliance with the ISO 10993-11:1996 standard. Microcyn™ was applied to the intact skin of 5 male and 5 female albino rabbits. The dose of Microcyn™ was 5050 mg / kg body weight, applied onto the dorsal area of the trunk. The test area subsequently was covered with a gauze. After 24 hours the area was rinsed with water to remove any test substance.

Conclusion: *observations made for 14 days, after application of the test substance revealed no signs of dermal irritation in any animals at any time. All subjected animals appeared normal for the time of the study and gross necropsy conducted at the termination of the study revealed no observable abnormalities. Based on this study, Microcyn™ is concluded not to generate dermal toxicity.* [Stillmeadow, Inc., United States of America]

Acute inhalation Toxicity

A test was executed in accordance with EPA OPPTS 870.1300 regulations to determine acute inhalation toxicity of Microcyn™ as required for compliance with the ISO 10993-11:1996 standard. Microcyn™ was applied as an aerosol to 5 male and 5 female albino rats. The dose of Microcyn™ was 2.16 mg / L air, applied to the rats by nasal inhalation for 4 consecutive hours.

Conclusion: *observations made for 14 days, after application of the test substance revealed no signs of pharmacologic or toxic activity. All subjected animals appeared normal for the time of the study and gross necropsy conducted at the termination of the study revealed no observable abnormalities. Based on this study, Microcyn™ is concluded to cause no toxic effects when inhaled.* [Stillmeadow, Inc., United States of America]

Safety and Efficacy of Treatment For Cutaneous Wound Study

A study was conducted with 16 rats to evaluate the local tolerability of Microcyn™ and its effects on the histopathology of wound beds in a model of full-thickness dermal wound healing. Masson's trichrome-stained sections and Collagen Type II stained sections of the Microcyn™ and saline-treated surgical wound sites were evaluated by a board-certified veterinary pathologist. The sections were assessed for the amount of Collagen Type II expression as a manifestiation of connective tissue proliferation, fibroblast morphology and collagen formation, presence of neoepidermis in cross section, inflammation and extent of dermal ulceration.

Conclusion: *the findings suggest that Microcyn™ was well tolerated in rats when administered under the conditions of this study. There were not treatment-related histopathologic lesions in the skin sections on either the left or the right sides (Microcyn™-treated and saline-treated respectively) There were no relevant histopathologic differences between the saline-treated and the Microcyn™ -treated wound sites, indicated that the Microcyn™ -treatment was well tolerated. There were no significant differences between Collagen Type II expression between the saline-treated and Microcyn™-treated wound sites including that the Microcyn™ does not have an adverse effect on fibroblasts or on collagen elaboration under the conditions of this study.*

Material compatibility

A corrosion test was performed in accordance with the requirements of ASTM G60-01 standard. The materials tested were: PVC, 303 Stainless Steel, 316 Stainless Steel, HA Aluminum, Titanium, Aluminum, Polyester, Teflon, Polypropylene, Natural Rubber, HDPE, Neoprene, Nylon, Silicone, Polycarbonate, Polyurethane, LDPE, Polysulfone, UHMWP and 416 Stainless Steel. Representative coupons were made from the test materials and daily brought into contact for 30 minutes with Microcyn™ on 20 consecutive days. During and at the end of the test, coupons are observed for visual appearance and weight.

Conclusions: *industrial metals like untreated Aluminum and 416 Stainless Steel, showed signs of corrosion after having been in contact with Microcyn™ for 20 days. All other materials maintained their visual appearance and their weight at constant levels. Based on the findings of this study Microcyn™ was determined compatible and not corrosive with all tested materials except Aluminum and 416 Stainless Steel. With the latter two, Microcyn™ is concluded to be similarly corrosive as water* [Micromed Laboratories Inc., United States of America]

Quality Control

Microcyn™ is manufactured according to the ISO 13485:2003 standard. All manufactured product lots are tested in-house for anti-microbial performance using the suspension method against Bacillus subtilis spores (a minimum reduction of 10^6 is the applicable requirement). Furthermore the product is tested for pH, ORP and free available chlorine levels as an in-process quality inspection.

Stability Tests

Microcyn™ has been shown to be stable and effective when aged. Product chemistry and antimicrobial efficacy for accelerated-aging have been tested in accordance with EPA guidelines. Based upon this data, the product has a shelf life of one (1) year.

MKT 41322.01 Rev. 2