OCULUS
Innovative Sciences

TOXICITY, EFFICACY, STABILITY AND CORROSION TEST RESULTS

MICROCYN™ TECHNOLOGY

TOXICITY STUDIES

SKIN IRRITATION

This study was conducted to assess the potential of Microcyn™ to produce dermal irritation and was conducted at Northview Pacific Laboratories, Inc, an ISO-certified lab located in California. Three New Zealand White rabbits each received 0.5mL of Microcyn™ applied directly to intact clipped skin sites. After a minimum of 4 hours of exposure to Microcyn, the test sites were observed at 1, 24, 48 and 72 hours. All test samples at each time interval showed no signs of erythema or edema formation. Therefore, it was concluded that the irritation response category of Microcyn is classified as negligible.

Zobair Musa, Study Director, *Northview Pacific Laboratories*

OCULAR IRRITATION

This study was conducted to assess the potential of Microcyn™ to produce ocular irritation and was conducted at Northview Pacific Laboratories, Inc. In this study, three New Zealand white rabbits each received 0.1mL of Microcyn applied directly into the right eye. The test sites were observed at 1, 24, 48 and 72 hours. All test samples at each time interval showed no signs of clinical observations or ocular irritation. Therefore, it was concluded that Microcyn™ did not cause a positive irritation response in the eyes of the test animals.

Zobair Musa, Study Director, *Northview Pacific Laboratories*

ACUTE ORAL TOXICITY

Microcyn™ was evaluated for its acute oral toxicity potential in albino rats when administered as a gavage dose at a level of 5000mg/kg. Three (3) female albino rats each received a volume of Microcyn of 4.98ml/kg. Each dose was administered using an appropriately sized syringe and stainless steel ball-tipped intubation needle. Observations for mortality and clinical/behavioral signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was sacrificed and subjected to gross necropsy. There was no mortality during the study in any of the test subjects. Body weight gain was unaffected by the administration of the test substance. All animals appeared normal for the duration of the study. The gross necropsy conducted at termination of the study revealed no observable abnormalities.

Janice O. Kuhn, PhD, DABT, *Stillmeadow, Inc.*

ACUTE DERMAL TOXICITY

Microcyn™ was evaluated for its dermal toxicity potential and relative skin irritancy when a single, undiluted dose of 5050mg/kg was applied to the intact skin of albino rabbits. Ten (10) rabbits each received a thin, uniform layer of Microcyn™ applied to the dorsal surface of the trunk. The test area was then covered with gauze and secured with non-irritating adhesive tape. After 24 hours, the wrappings were removed and the test areas were gently washed to remove any test substance. Observations for mortality and clinical/behavioral signs of toxicity were made at least three times on

the day of dosing (Day 0) and at least once thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. Observations for dermal irritation were made at approximately 60 minutes after removal of wrappings, and on Days 4, 7, 11 and 14. On Day 14 after dosing, each animal was sacrificed and subjected to gross necropsy. There was no mortality during the study in any of the test subjects. Body weight gain was unaffected by the administration of the test substance, with the exception of one male and one female that lost weight, and one female that failed to gain weight between Days 7 and 14. All test subjects appeared normal for the duration of the study. There were no signs of dermal irritation in any animals at any time during the study. The gross necropsy conducted at termination of the study revealed no observable abnormalities.

Janice O. Kuhn, PhD, DABT, *Stillmeadow, Inc.*

ACUTE INHALATION TOXICITY

Microcyn™ was evaluated for its acute inhalation toxicity potential in ten (10) albino rats. The test subjects were individually housed in polycarbonate exposure test tubes that were then inserted into a 500L stainless steel nose-only inhalation chamber for four (4) hours once a 99% concentration was attained. Each test subject was exposed to an aerosol generated from the undiluted test substance at a level of 2.16 mg/L. Observations for mortality and signs of pharmalogic and/or toxicologic effects were made frequently on the day of exposure and at least once daily thereafter for 14 days (Day 0 is day of exposure). Individual body weights were recorded just prior to the inhalation exposure and on Days 7 and 14. On Day 14 after exposure, each animal was euthanized and subjected to gross necropsy and all abnormalities were recorded. There was no mortality during the study. As indicated by the date, the acute inhalation LC_{50} for Microcyn™ is greater than 2.16mg/L. Body weight gain was unaffected by the administration of the test substance, except for one male that failed to gain weight between Days 0 and 7. Prominent in-life observations included very-slight to slight activity decreases and piloerection in both sexes at 4.5 and 6 hours. Animals were asymptomatic by Day 1. The gross necropsy conducted on each animal at termination of the study revealed no observable abnormalities. Therefore, Microcyn™ passed the acute inhalation limit test of >2mg/L for 4 hours.

Lori Carter, BA, *Stillmeadow, Inc.*

DERMAL SENSITIZATION

A skin sensitization study was conducted on thirty (30) guinea pigs to determine if Microcyn™ produced a sensitizing reaction. Twenty (20) test subjects (Group II) were treated with 0.4 mL of undiluted Microcyn™ administered beneath a surgical gauze patch and then securely taped. Each animal was then placed in a restrainer for approximately six (6) hours. The animals were treated once weekly for three (3) weeks, for a total of three (3) treatments. At the end of the exposure period, the animals were removed from the restrainers and the wrappings and patches were removed. After a two-week rest period, all animals (Group II and ten (10) animals in the untreated control Group I) were challenged (the "challenge treatment") at a virgin test site with an application of 0.4 mL of undiluted test substance administered in the same manner as the induction treatments of Group II. Observations for skin reactions at each test site were made approximately 24 hours and 48 hours after the first treatment and at 48 hours after the challenge treatment. Microcyn™ produced no irritation in Group I after the single treatment at challenge. Likewise, Microcyn™ produced no irritation in Group II after the challenge treatment and therefore did not elicit a sensitizing reaction in guinea pigs. Therefore, Microcyn™ can be characterized as a non-sensitizer.

Janice O. Kuhn, PhD, DABT, *Stillmeadow, Inc.*

EFFICACY STUDIES

SPORICIDAL SCREEN

Fifteen test samples were challenged with *Bacillus subtilis* mixed with a 5% organic load. Test samples were prepared in accordance with the EPA-recognized AOAC sporicidal test method. Microcyn™ passed the sporicidal test when the porcelain penicylinders were exposed for 15 minutes at 20˚C. All samples exhibited zero growth.

Felicia L. Sellers *MICROBIOTEST, Inc*

BACTERICIDAL

This test uses *Pseudomonas aeruginosa* (ATCC #15442), *Staphylococcus aureus* (ATCC #6538) and *Salmonella choleraesuis* (ATCC #10708). Three lots of Microcyn™ were tested on 60 carriers per lot per organism. A film of bacterial cells dried on a surface of stainless steel carriers was exposed to the test substance. Following exposure, the carriers were transferred to vessels containing neutralizing subculture medium (Letheen Broth + 0.1% Sodium Thiosulfate) and assayed for survivors. Test samples were incubated at 35˚± 2˚C for 48 hours. This test was conducted in accordance with the EPA-recognized AOAC Use-Dilution method. For each lot of 60 carriers, EPA requirements specify that a minimum of 59 must show no growth. This requirement was met for each of these three organisms (see below).

Test Substance	Test Organism	Exposure Time	Sample Dilution	Total # of Carriers Exposed	Total # of Carriers Showing Growth**
Microcyn™	*P. aeruginosa*	10 minutes	RTU	180	0
Microcyn™	*S. aureus*	10 minutes	RTU	180	2
Microcyn™	*S. choleraesuis*	10 minutes	RTU	180	1

*RTU=Ready to Use **Number of carriers showing growth of the test organism*

David Rottjakob M.T. ATS Labs.

BACTERICIDAL

This test was conducted using *Escherichia coli* 0157;H7 (ATCC #35150) was conducted on two lots of Microcyn™ using 10 carriers per lot. A 5% organic load was added to each test sample. The carriers were exposed to Microcyn™ for 1 minute and incubated for 48 hours at 35˚± 2˚C. This test was conducted in accordance with the EPA-recognized AOAC Use-Dilution method. All results showed zero growth after one-minute exposure time.

David Rottjakob M.T. *ATS Labs.*

MRSA

This test uses *Staphylococcus aureus* (ATCC #33592). Sterile penicylinders were immersed for 15 minutes in a 48-54 hour-old broth culture of the test organism at the ratio of 1 carrier per 10ml. The penicylinders were then dried on filter paper in a sterile petri dish at 36°±1°C for 40 minutes at 71.2% RH. Ten (10) contaminated and dried carriers were then transferred to individual tubes containing 10ml of Microcyn™ and exposed for 10 minutes at 20°±1°C. The carriers were then transferred to a neutralizing solution and incubated for 48 hours at 36°±1°C. At the end of this time, each carrier was examined for the presence or absence of visible growth. Appropriate controls were conducted and all met the appropriate acceptance criteria. Two lots of Microcyn™ were used. No growth was observed on any of the twenty (2 lots, 10 carriers) subcultures after 10-minute exposure to Microcyn™.

David Rottjakob M.T. *ATS Labs.*

VRE

This test uses *Enterococcus faecalis* Vancomycin Resistant (ATCC #51299). Sterile penicylinders were immersed for 15 minutes in a 48-54 hours-old broth culture of the test organism at the ratio of 1 carrier per 10ml. The penicylinders were then dried on filter paper in a sterile Petri dish at 36°±1°C for 40 minutes at 76.1% RH. Ten (10) contaminated and dried carriers were then transferred to individual tubes containing 10ml of Microcyn™ and exposed for 10 minutes at 20°±1°C. The carriers were then transferred to a neutralizing solution and incubated for 48 hours at 36°±1°C. At the end of this time, each carrier was examined for the presence or absence of visible growth. Appropriate controls were conducted and all met the appropriate acceptance criteria. Two lots of Microcyn™ were used. No growth was observed on any of the twenty (2 lots, 10 carriers) subcultures after 10-minute exposure to Microcyn™.

David Rottjakob M.T. *ATS Labs.*

MYCOBACTERIUM/TUBERCULOCIDAL

This test uses *Mycobacterium bovis* (OT 105401) according to Tuberculocidal Quantitative Suspension (EPA) protocol. This was conducted under EPA Good Laboratory Practice Regulations (GLP). A 5% organic load (fetal bovine serum) was added to the organism suspension. A single tube was inoculated to the organism followed by exposure to Microcyn™ for 5 minutes. Samples were incubated for 20 days at 35˚± 2˚C. No growth after 5-minute contact time.

David Rottjakob M.T. *ATS Labs.*

AOAC AVAILABLE CHLORINE IN DISINFECTANTS

This test uses *Salmonella typhi* (ATCC #6539). A 0.05mL aliquot of the test culture suspension was added to the test substance and control NaOCI solutions previously equilibrated to 37˚C. One minute after addition of the test organism, one loopful of each medicated culture was transferred to the subculture medium. Each tube was then challenged with an additional 0.05mL aliquot of the test culture 30 seconds after subculturing. This process was repeated for a total of 10 subcultures for each lot and control. The neutralized subcultures were incubated for 48 hours at 35˚± 2˚C. Following incubation, the subcultures were examined for the presence or absence of visible growth. This test was conducted in accordance with the EPA-recognized AOAC Use-Dilution method.

Test Substance	Test Organism	Concentration or Lot	Subculture Series 1	2	3	4	5	6	7	8	9	10
NaOCl Control	*S. typhi*	200 ppm	0	0	0	0	0	0	0	+	+	+
NaOCl Control	*S. typhi*	100 ppm	0	0	0	0	+	+	+	+	+	+
NaOCl Control	*S. typhi*	50 ppm	0	0	+	+	+	+	+	+	+	+
Microcyn™	*S. typhi*	Lot # 090803	0	0	0	0	0	0	0	+	+	+

+=Growth of Organism 0=No growth of organism.

The subcultures of positive broths (tubes showing growth) demonstrated pure cultures of the test organism.

David Rottjakob, M.T. *ATS labs*

Therefore, Microcyn™ with available chlorine levels of approximately 60 ppm has demonstrated germicidal equivalence to a control sample with 200 ppm available chlorine. Salmonella typhi was used as the challenge microorganism.

VIRUCIDAL

Two lots of Microcyn™ were tested to determine efficacy when exposed to the Human Immunodeficiency Virus Type I, Strain HTLV-IIIB in the presence of 5% organic soil load. For each lot of test substance, separate dried virus films were exposed to 2.0 mL of the use dilution for ten minutes at 21C. The filtrate (10-1 dilution) was then tittered by 10-fold serial dilutions and assayed for infectivity. The reduction in viral titer was ≥ 3.75 log10 for both lots. Therefore, Microcyn™ demonstrated complete inactivation of the HIV-1 virus at a 10-minute exposure time.

Dilution	Dried Virus Control (Group A)	HIV-1 +Lot P120104 (Group B)	HIV-1 +Lot P130104 (Group B)
Cell Control	0000	0000	0000
10^{-1}	++++	TTTT	TTTT
10^{-2}	++++	0000	0000
10^{-3}	++++	0000	0000
10^{-4}	++++	0000	0000
10^{-5}	++00	0000	0000
10^{-6}	+000	0000	0000
10^{-7}	0000	0000	0000
$TCID_{50}$/0.2mL	$\leq 10^{5.25}$	$\leq 10^{1.5}$	$\leq 10^{1.5}$

T=Toxic +=Cytopathic effect 0=No cytopathic effect

Mary J. Miller, M.T. *ATS Laboratories*

VIRUCIDAL SCREEN

Microcyn™ was tested against Canine parvovirus using an exposure period of 15 minutes. The test substance was combined with a 1% organic load. Test samples were incubated for 7 days at 36˚-38˚C. Microcyn™ demonstrated complete inactivation of the virus following a 15-minute exposure time. This test was in accordance with key segments of the EPA-recognized ASTM Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces.

David Rottjakob, M.T. *ATS labs*

STABILITY STUDIES

Microcyn™ has been shown to be stable and effective when aged. Product chemistry and antimicrobial efficacy for both real-time and accelerated-aging have been tested in accordance with EPA, FDA (CDRH) and ICH guidelines. Based upon this data, the product has a shelf life of one (1) year. Accelerated product shelf life studies at 50°C support a 2-year product shelf life. Additional real-time data is currently being gathered to support 2-year real-time shelf life.

CORROSION STUDIES

Good Lab Practices (GLP) were utilized to document the chemical resistance testing of various materials repeatedly exposed to Microcyn™. The corrosion test was performed in accordance with the requirements of ASTM standards G60 and G1. Test coupons of materials were representative of what would be expected if these materials were in service. Coupons were cleaned to remove contaminants in appropriated methods that would not affect coupon's material, mechanical and physical properties. Six specimens and one control of each material were tested. Acceptance criteria included that the weight and visual appearance of each coupon should be equivalent after testing. 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.

Conclusion: All plastics and elastomers have met the stated acceptance criteria set forth in the ASTM standards by maintaining equivalent weight and appearance throughout the study. Any instance where weight gain occurred has been successfully attributed to either the humid environment or the inherent properties of the material involved. Based on the data, Microcyn™ is compatible with all the materials tested and is not considered corrosive. The exceptions are Aluminum and 416 Stainless Steel, which are prone to corrosion due to exposure to moisture in the environment.

QUALITY CONTROL

Microcyn™ is manufactured under Good Manufacturing Practices (cGMP) requirements set forth by the U.S. Food and Drug Administration (FDA) and ISO 13485 standards. All manufacturing lots are tested for anti-microbial effectiveness using suspension method (Bacillus subtillis spores to document a 10^6 reduction) pH, ORP, and free available chlorine levels.

For more information on the non-toxic Microcyn™ technology, visit our web site at www.oculusis.com.

UNITED STATES:

Oculus Innovative Sciences, 1129 North McDowell Blvd.,
Petaluma, California 94954 USA
tel: (707) 283-0550, **fax:** (707) 283-0551, **website:** www.oculusis.com

INTERNATIONAL:

Manufacturing and distribution facilities in
Morelia, Mexico and Sittard, The Netherlands

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