



TEST DATA FOR ZGUARD INGREDIENTS

EFFICACY TEST DATA

Efficacy Test Data

Disinfecting Detergent – (EPA Manufacturing Facility Reg. No. 82859)

VIRUCIDAL DATA

Testing Methods

* U.S. E.P.A. Pesticide Assessment Guidelines, Subdivision G: Product Performance, 1982, Section 91-30, pp. 72-76.

Virucide Assay (EPA, Federal Register 10, No. 123, 6/25/75, p. 26836). Protocols for Testing the Efficacy of Disinfectants against Hepatitis B Virus (HBV) (EPA, Federal Register, Vol., 65, No. 166, 8/25/2000, p. 51828).

Protocol for Testing Disinfectants against Hepatitis C Virus using Bovine Viral Diarrhea Virus as approved by the U.S. EPA on August 15, 2002.

Test Conditions: 2 oz. per gallon of water dilution, 10 minute contact time, tested in the presence of serum glass petri dish substrates

Results

Test Organism Sample: Titer Reduction

Adenovirus Type 2 AB 3.0 log 10 > 3.0 log 10

*Avian Influenza A Virus (H3N2) (Avian Reassortant) (ATCC VR-2072) AB >3.5 log 10 > 3.5 log 10

*Avian Influenza Virus, Type A (Turkey /WIS /66) (H9N2) AB >4.5 log 10 > 4.5 log 10
Bovine Viral Diarrhea Virus (BVDV) AB 6.1 log 10 > 3.8 log 10 > 4.5 log 10

*Feline Calicivirus (FCV) AB 5.79 log 10 > 6.06 log 10

Hepatitis B Virus (HBV) (Duck Hepatitis B Virus-DHBV) AB 4.5 log 10 > 4.5 log 10
Hepatitis C Virus (HCV) (Bovine Viral Diarrhea Virus-BVDV) AB 6.1 log 10 > 3.8 log 10
Herpes Simplex Type I (Sabin) AB >4.0 log 10 > 3.7 log 10

*Human Coronavirus (ATCC VR-740, strain 229E) AB >3.0 log 10 > 3.0 log 10

*Human Immunodeficiency Virus, HTLV-II_{RF}, strain of HIV-I (associated with AIDS)
AB >3.0 log 10 > 3.0 log 10

Influenza A:- (Japan 305/57) AB >6.5 log 10 > 6.0 log 10

*Norovirus (Norwalk Virus) (FCV) AB 5.79 log 10 > 6.06 log 10

*SARS Associated Coronavirus (ZeptoMetrix) AB 4.03 log w 4.03 log 10
Vaccinia (Wyeth) AB >3.5 log 10 > 3.5 log 10

Conclusion

Under the conditions of this investigation, this Detergent/Disinfectant was virucidal for Adenovirus Type 2, Avian Influenza A Virus (H3N2), Avian Influenza Virus Type A (H9N2), Bovine Viral Diarrhea Virus (BVDV), Feline Calicivirus (FCV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Herpes Simplex Type I (Sabin), Human Coronavirus, Human Immunodeficiency Virus (HIV-1), Influenza A2 (Japan 305/57), Coronavirus (Norwalk Virus), SARS Associated Coronavirus and Vaccinia (Wyeth) according to criteria established by the U.S. Environmental Protection Agency for registration and labeling of a disinfectant product as a virucide.

MILDEW FUNGISTATIC DATA

Testing Method

Hard Surface Mildew Fungistatic Test (Unofficial Protocol, 10/27/76)
Test Organism: *Aspergillus niger* (ATCC 6275)
Test Conditions: tile substrates

Sample Dilution
of Exposed Tiles
of Tiles Showing Growth
This Detergent/Disinfectant oz/gal IO 0

Control – IO 10

Conclusion

Under the conditions of this investigation, this Detergent/Disinfectant was fungistatic for *Aspergillus niger* according to criteria established by the U.S. Environmental Protection Agency for registration and labeling of a disinfectant product as a fungistat.

FUNGICIDAL DATA

Test Method
AOAC Fungicidal Test
Test Organism: *Trichophyton mentagrophytes* (ATCC 9533)

Test Conditions: 2 oz/gal dilution 5% organic soil load
20°C exposure temperature

Results

Exposure Time (min.) vs. Growth Sample 5 10 15 A

B+ + 0 0 0 0

Conclusion

Under the conditions of this investigation, this Detergent/Disinfectant was fungicidal for Trichophyton mentagrophytes according to criteria established by the U.S. Environmental Protection Agency for registration and labeling of a disinfectant product as a fungicide .

DISINFECTION DATA

Test Method

AOAC Use Dilution

Test Conditions: 5% organic soil load, 10-minute contact time, stainless steel carrier substrates, 20°C exposure temperature, 2 oz/gal dilution

No. of Carriers

Test Organism Sample Exposed Positive Staphylococcus aureus (ATCC 6538) A B

C 60 60 60 0 0 0

Salmonella choleraesuis (ATCC 10708) A B C 60 60 60 0 0 0

Pseudomonas aeruginosa (ATCC 15442) A B C 60 60 60 0 0 0

Brevibacterium ammoniagenes (ATCC 6871) A B 10 10 0 0

Enterobacter aerogenes (ATCC 13048) A B 10 10 0 0

Escherichia coli (ATCC 11229) A B 10 10 0 0

Klebsiella pneumoniae (ATCC 4352) A B 10 10 0 0

Listeria monocytogenes (ATCC 984) A B 10 10 0 0

Methicillin resistant Staphylococcus aureus (MRSA) (ATCC 33593) A B 10 10 0 0

Salmonella schottmuelleri (ATCC 8759) A B 10 10 0 0

Shigella dysenteriae (ATCC 12180) A B 10 10 0 0

Streptococcus faecalis (ATCC 10541) A B 10 10 0 0

Streptococcus pyogenes (Clinical-Flesh Eating Strain, BIRD M3) A B 10 10 0 0

Streptococcus salivarius (ATCC 9222) A B 10 10 0 0

Vancomycin intermediate resistant Staphylococcus aureus (VIRSA) A B 10 10 0 0

Conclusion

Under the conditions of these investigations, this Detergent/Disinfectant demonstrated disinfectant activity against *Staphylococcus aureus*, *Salmonella choleraesuis*, *Pseudomonas aeruginosa*, *Brevibacterium ammoniagenes*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Usteria monocytogenes*, Methicillin resistant *Staphylococcus aureus* (MRSA), *Salmonella scholftmue/leri*, *Shigella dysenteriae*, *Streptococcus faecalis*, *Streptococcus pyogenes* (Clinical – Flesh Eating Strain, BIRD M3), *Streptococcus salivarius* and Vancomycin intermediate resistant *Staphylococcus aureus* (VIRSA) according to criteria established by the U. S. Environmental Protection Agency for registration and labeling of a disinfectant product as a bactericide.

SANITIZATION DATA

Test Method

AOAC Germicidal and Detergent Sanitizing Action of Disinfectants

Test Conditions: 200 ppm active quaternary 2 o-z/3.5 gal dilution

Results

TOTAL BACTERIAL COUNTS /

% KILL vs. EXPOSURE TIME

Synthetic Hard Water 30 seconds

60 seconds

Test Organism Sample (ppm) TBC • % Kill TBC • % Kill

Staphylococcus aureus

(ATCC 6538)

A B C

250

250

250

1120

106 5

1 275

99.999

99.999

99.999

65

70

185

99.999

99.999

99.999

Escherichia coli (ATCC 1 12 29) A

B C 300

300

300

990

1215

1460

99.999

99.999

99.999

65

80

190

99.999

99.999

99.999

• TBC = Total Bacterial Count, cfwml t % Kill calculated based on initial inoculum control count of 75×10^5 x 10⁶ cfu/ ml.

Conclusion

Under the conditions of these investigations, this Detergent/Disinfectant demonstrated sanitizing activity against Staphylococcus aureus and Escherichia coli according to criteria established by the U. S. Environmental Protection Agency for registration and labeling of a disinfectant product as a sanitizer.

DERMAL SKIN TEST DATA

DERMAL IRRITATION TESTING DATA

Summary of Dermal Irritation Testing on this disinfectant/detergent 01/10/07

The Method used in Protocol Design was the Modified Draize method as described in OECD Guidelines for the Testing of Chemicals, Sec. 404, Paris 1981 (revised: 1992)

In each animal, the sum of the skin values for erythema at 1, 24, 48 and 72 hours for exposed areas was added to the similar sum of the values for oedema formation. The primary irritation index for each animal was the sum of the two summary values divided by 3 (i.e. the average of the three readings). The primary irritation score and its standard deviation are the mean value and standard deviation of the primary irritation indices of the three animals.

A 0.5 mL portion of the test disinfectant/detergent was topically applied to the intact skin of a group of three rabbits by patch application. The test article stayed in contact with the skin for a 4-hour period.

The test sites were evaluated at 1, 24, 48, and 72 hours following the exposure period. The test article showed no erythema or oedema on all animals at one hour after the exposure period. At 24 hours after the exposure period, no erythema was observed on all animals. At 72 hours after the exposure period, no erythema was observed on all animals.

NOTE: this disinfectant/detergent was applied at pure, undiluted strength. Classification of primary irritation scores:

0 – 0.9	Non-Irritant
1.0 – 1.9	Very Mild Irritant
2.0 – 3.9	Mild Irritant
4.0 – 5.9	Moderate Irritant
6.0 – 8.0	Severe Irritant

Based on these results, the test article was classified as follows: Primary Irritation Score 0.3 ± 0.1

Classification: Non- Irritant

Based on the above findings, the test article is not classified according to the Transportation of Dangerous Goods Act.

Based on the above findings, the test article is classified as NON- IRRITANT according to OSHA, US D.O.T. and the Canadian Transportation of Dangerous Goods Act testing protocols thus requires no PPE's as per 29CFR.

DERMAL EYE TEST DATA

Summary of Eye Irritation Testing /C PR 1500.42 on this disinfectant/detergent 11/17 /07

Herein referred to as this disinfectant/detergent

The Method used in Protocol Design was the Draize method as described in OECD Guidelines for the Testing of Chemicals, Sec. 405, OPPTS 798.4500 Primary Eye irritation, OPP 81-4 Acute Eye Irritation-Rabbit, and EPA report 540/09-82, 1982.

Six albino rabbits shall be used in accordance with CFR 1500.42. In each animal, the test material shall be placed into one eye of each rabbit. The eyelids shall then gently be held together for one second and then the rabbit shall be released. The grade of ocular reaction is recorded at 1, 24, 48 and 72 hours. The sum of the grade of ocular reaction shall then be added. The primary irritation index for each animal was the sum

of the two summary values divided by 6 (i.e. the average of the three readings). The primary irritation score and its standard deviation are the mean value and standard deviation of the primary irritation indices of the three animals.

A 0.1 mL portion of this disinfectant/detergent was topically applied to the intact eyes of a group of six rabbits by placing the test material with a sterile dropper into the conjunctival sac of one eye of each rabbit by gently pulling the lower lid away from the eyeball.

The test sites were evaluated at 1, 24, 48, and 72 hours following the exposure period. The test article showed no ocular reaction on all rabbits at 24 hours after the exposure period. At 1 hour after exposure, no ulcerations or opacity were observed.

However, slight redness was apparent in 1 of the 6 rabbits. At 24 hours after the exposure, no ulcerations or opacity were observed. The 1 rabbit that showed slight redness had essentially recovered 100% at this testing interval. At 48 hours after the exposure, no ulcerations or opacity were observed. At 72 hours after the exposures, no ulcerations or opacity were observed.

NOTE: this disinfectant/detergent was applied at pure, undiluted strength. Classification of primary irritation scores:

0-7.0	Non-Irritant
7.1-5.0	Practically Non-Irritating
15.1-25.0	Slightly Irritating
25.1-50.0	Moderately Irritating
50.1-110.0	Severely Irritating /Corrosive

References:

(1) Buehler, E.Y. and Newmann, E.A. A comparison of Eye Irritation in Monkeys and Rabbits. Toxicology and Applied Pharmacology 6:701-710 (1964)

(2) Draize, J.H. et al. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. Journal of Pharmacology and Experimental Therapeutics. 83-377-390 (19,/.4)

Technology Stabilized Non Spore Microbes

This disinfectant/detergent's patented technology utilizes the ability to stabilize live vegetative microbes in a liquid form providing it with unique benefits vs. traditional spore based systems, thereby allowing our clients the opportunity to market the next generation of microbial based products.

In addition to ZGUARD's safety with concern to the end users accidental skin and eye contact and its superior efficacy test results, ZGUARD also out performs competing formulas in the following environments:

It will easily and effectively degrade the following but not limited to:

Petroleum hydrocarbons

Fats Oils Greases

Stubborn organic compounds

Human and Animal feces

Unique benefits:

No germination time required, goes to work immediately (conventional spore technology requires germination time of 12-24 hrs)

More complete degradation (metabolism) resulting in a faster elimination and reduction of odors

Completely degrades hydrocarbons to carbon dioxide and water

Consistent Lipase production under most all field conditions

Significantly reduces BOD, COG, and FOG

Excellent performance in a varying range of pH and temperature

Most stable in the industry, no reduction of cfu's for 12 months+ in both concentrated form and at dilutions up to 10:1

Performs equally under aerobic and anoxic conditions Salmonella free, Nonpathog