

**Biodet.**

Laboratory 01707 284522  
Email Biodet@herts.ac.uk

John Ware  
Eco Green Cleaners Ltd  
82 Gordian Way  
Stevenage Hertfordshire  
SG2 7QH

Ref: EGC/12/1992  
Date: 5<sup>th</sup> July 2012  
Log No: 1992

**Determination of the bactericidal activity against *Legionella pneumophila***

The tests were performed at the Biodet laboratories, University of Hertfordshire.  
Tests were conducted for Environmental Manufacturing Solutions, LLC / Eco Green Cleaners Ltd.

**Identification of the sample**

Products: Xtreme, Agri-Complete & Blow-out  
Batch number: Xtreme 3767-11, Agri-Complete 3766-11, Blow-out 3665-11  
Expiry date: Unknown  
Manufacturer: Environmental Manufacturing Solutions, LLC  
Date of delivery: June 2012  
Storage conditions: Room temperature  
Active substance: Not indicated

**Test method and its validation**

Method: Membrane filtration  
Neutralizer: Tap water (sterile)

**Experimental conditions**

Period of analysis: 21 June 2012 to 28 June 2012  
Appearance of the products: Colourless clear liquids  
Product test concentration: Agri-Complete & Xtreme at 1.56%. Blowout at 10%  
Test temperatures: 20 C & 30C  
Contact times: 60 minutes  
Interfering substances: 0.000 5 w/v yeast extract  
Product diluent: Hard water & buffered ferrous hard water  
Stability of the test mixtures: Stable  
Temperature of incubation: 36C +/- 1C  
Counting procedure: Membrane filtration  
Bacterial strain used: NCTC 11192

**Test results**

See tables A.1 & A.2 to F.1 & F2

**Conclusion**

According to EN 13623 (2010), the June 2012 batch of **Xtreme**, Agri-Complete and Blowout, when diluted at 1.56%, 1.56% and 10% respectively in hard water and buffered ferrous hard water, possesses bactericidal activity against the reference strain of *Legionella pneumophila* serogroup 1, NCTC 11192.



R SMITH  
LAB DIRECTOR

5<sup>th</sup> July 2012  
DATE

**Table A.1 Verification of the methodology and validation of membrane filtration for the test concentration of 1.56% Xtreme using hard water**

Test organism	Colony counts (Vc-values per 0.1ml sample)				
	Bacterial test suspension N	Neutralization test suspension N <sub>v</sub>	Neutralizer toxicity control B	Method validation C	Experimental conditions A
<i>Legionella pneumophila</i>	33, 30 at dilution of 10 <sup>-6</sup> (N = 3.15 x 10 <sup>8</sup> )  N <sub>0</sub> = 3.15 x 10 <sup>7</sup>	33, 30  (N <sub>v</sub> = 315)  N <sub>v0</sub> = 31.5	24  B=24	27, 32  (C = 29.5)	>165, >165  (A = >165)

**Table A.2 Test Results Xtreme in hard water at 20 C**

Test organism	Membrane filtration counts of test mixture at test concentration and contact time indicated cfu/ml		
	1.56% 60 minutes	0.52% 60 minutes	0.00156% 60 minutes
Vc – values (N <sub>a</sub> <sup>0</sup> )	<14, <14	<14, <14	>165, >165
<i>Legionella pneumophila</i> (N <sub>a</sub> )	<140	<140	>1650
Calculation of lg reduction counts	7.50 – 2.15 = 5.35	7.50 – 2.15 = 5.35	7.50 – 3.22 = 4.28
lg R = lg N <sub>0</sub> – lg N <sub>a</sub>			

### Conclusion

The product Xtreme at 1.56% (1/64) was found to be bactericidal at 20 C under the conditions of EN 13623 (2010).

**Table B.1 Verification of the methodology and validation of membrane filtration for the test concentration of 1.56% Xtreme using buffered ferrous hard water**

Test organism	Colony counts (Vc-values per 0.1ml sample)				
	Bacterial test suspension N	Neutralization test suspension N <sub>v</sub>	Neutralizer toxicity control B	Method validation C	Experimental conditions A
<i>Legionella pneumophila</i>	24, 27 at dilution of 10 <sup>-6</sup> (N = 2.55 x 10 <sup>8</sup> )  N <sub>0</sub> = 2.55 x 10 <sup>7</sup>	24, 27  (N <sub>v</sub> = 255)  N <sub>v0</sub> = 25.5	27, 26  B=26.5	27, 32  (C = 29.5)	>165, >165  (A = >165)

**Table B.2 Test Results Xtreme in buffered ferrous hard water at 30 C**

Test organism	Membrane filtration counts of test mixture at test concentration and contact time indicated cfu/ml		
	1.56% 60 minutes	0.52% 60 minutes	0.00156% 60 minutes
Vc – values (N <sub>a</sub> <sup>0</sup> )	<14, <14	<14, <14	>165, >165
<i>Legionella pneumophila</i> (N <sub>a</sub> )	<140	<140	>1650
Calculation of lg reduction counts	7.41 – 2.15 = 5.26	7.41 – 2.15 = 5.26	7.41 – 3.22 = 4.19
lg R = lg N <sub>0</sub> – lg N <sub>a</sub>			

### Conclusion

The product Xtreme at 1.56% (1/64) was found to be bactericidal at 30 C under the conditions of EN 13623 (2010).



**Table C.1 Verification of the methodology and validation of membrane filtration for the test concentration of 1.56% Agri-Complete using hard water**

Test organism	Colony counts (Vc-values per 0.1ml sample)				
	Bacterial test suspension N	Neutralization test suspension N <sub>v</sub>	Neutralizer toxicity control B	Method validation C	Experimental conditions A
<i>Legionella pneumophila</i>	33, 30 at dilution of 10 <sup>-6</sup> (N = 3.15 x 10 <sup>8</sup> )  N <sub>0</sub> = 3.15 x 10 <sup>7</sup>	33, 30  (N <sub>v</sub> = 315)  N <sub>v0</sub> = 31.5	24  B=24	26  (C = 26)	>165, >165  (A = >165)

**Table C.2 Test Results Agri-Complete in hard water at 20 C**

Test organism	Membrane filtration counts of test mixture at test concentration and contact time indicated cfu/ml		
	1.56% 60 minutes	0.52% 60 minutes	0.00156% 60 minutes
Vc – values (N <sub>a</sub> <sup>0</sup> )	<14, <14	<14, <14	>165, >165
<i>Legionella pneumophila</i> (N <sub>a</sub> )	<140	<140	>1650
Calculation of lg reduction counts	7.50 – 2.15 = 5.35	7.50 – 2.15 = 5.35	7.50 – 3.22 = 4.28
lg R = lg N <sub>0</sub> – lg N <sub>a</sub>			

**Conclusion**

The product Agri-Complete at 1.56% (1/64) was found to be bactericidal at 20 C under the conditions of EN 13623 (2010).

**Table D.1 Verification of the methodology and validation of membrane filtration for the test concentration of 1.56% Agri-Complete using buffered ferrous hard water**

Test organism	Colony counts (Vc-values per 0.1ml sample)				
	Bacterial test suspension N	Neutralization test suspension N <sub>v</sub>	Neutralizer toxicity control B	Method validation C	Experimental conditions A
<i>Legionella pneumophila</i>	24, 27 at dilution of 10 <sup>-6</sup> (N = 2.55 x 10 <sup>8</sup> )  N <sub>0</sub> = 2.55 x 10 <sup>7</sup>	24, 27  (N <sub>v</sub> = 255)  N <sub>v0</sub> = 25.5	27, 26  B=26.5	27, 32  (C = 29.5)	>165, >165  (A = >165)

**Table D.2 Test Results Agri-Complete in buffered ferrous hard water at 30 C**

Test organism	Membrane filtration counts of test mixture at test concentration and contact time indicated cfu/ml		
	1.56% 60 minutes	0.52% 60 minutes	0.00156% 60 minutes
Vc – values (N <sub>a</sub> <sup>0</sup> )	<14, <14	<14, <14	>165, >165
<i>Legionella pneumophila</i> (N <sub>a</sub> )	<140	<140	>1650
Calculation of lg reduction counts	7.41 – 2.15 = 5.26	7.41 – 2.15 = 5.26	7.41 – 3.22 = 4.19
lg R = lg N <sub>0</sub> – lg N <sub>a</sub>			

## Conclusion

The product Agri-Complete at 1.56% (1/64) was found to be bactericidal at 30 C under the conditions of EN 13623 (2010).

**Table E.1 Verification of the methodology and validation of membrane filtration for the test concentration of 10% Blow-out using hard water**

Test organism	Colony counts (Vc-values per 0.1ml sample)				
	Bacterial test suspension N	Neutralization test suspension N <sub>v</sub>	Neutralizer toxicity control B	Method validation C	Experimental conditions A
<i>Legionella pneumophila</i>	33, 30 at dilution of 10 <sup>-6</sup> (N = 3.15 x 10 <sup>8</sup> )  N <sub>0</sub> = 3.15 x 10 <sup>7</sup>	33, 30  (N <sub>v</sub> = 315)  N <sub>v0</sub> = 31.5	24  B=24	27, 31  (C = 29)	>165, >165  (A = >165)

**Table E.2 Test Results Blow-out in hard water at 20 C**

Test organism	Membrane filtration counts of test mixture at test concentration and contact time indicated cfu/ml		
	10% 60 minutes	3.33% 60 minutes	0.01% 60 minutes
Vc – values (N <sub>a</sub> <sup>0</sup> )	<14, <14	42, 41	>165, >165
<i>Legionella pneumophila</i> (N <sub>a</sub> )	<140	415	>1650
Calculation of lg reduction counts	7.50 – 2.15 = 5.35	7.50 – 2.62 = 4.88	7.50 – 3.22 = 4.28
lg R = lg N <sub>0</sub> – lg N <sub>a</sub>			

## Conclusion

The product Blow-out at 10% was found to be bactericidal at 20 C under the conditions of EN 13623 (2010).

**Table F.1 Verification of the methodology and validation of membrane filtration for the test concentration of 10% Blow-out using buffered ferrous hard water**

Test organism	Colony counts (Vc-values per 0.1ml sample)				
	Bacterial test suspension N	Neutralization test suspension N <sub>v</sub>	Neutralizer toxicity control B	Method validation C	Experimental conditions A
<i>Legionella pneumophila</i>	24, 27 at dilution of 10 <sup>-6</sup> (N = 2.55 x 10 <sup>8</sup> )  N <sub>0</sub> = 2.55 x 10 <sup>7</sup>	24, 27  (N <sub>v</sub> = 255)  N <sub>v0</sub> = 25.5	27, 26  B=26.5	27, 31  (C = 29)	>165, >165  (A = >165)

**Table F.2 Test Results Blow-out in buffered ferrous hard water at 30 C**

Test organism	Membrane filtration counts of test mixture at test concentration and contact time indicated cfu/ml		
	10% 60 minutes	3.33% 60 minutes	0.01% 60 minutes
Vc – values (N <sub>a</sub> <sup>0</sup> )	<14, <14	>165, >165	>165, >165
<i>Legionella pneumophila</i> (N <sub>a</sub> )	<140	>1650	>1650
Calculation of lg reduction counts	7.41 – 2.15 = 5.26	7.41 – 3.22 = 4.19	7.41 – 3.22 = 4.19
lg R = lg N <sub>0</sub> – lg N <sub>a</sub>			

### Conclusion

The product Blow-out at 10% was found to be bactericidal at 30 C under the conditions of EN 13623 (2010).



# TEST DATA FOR XTREME

## EFFICACY TEST DATA

### *Xtreme as a 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 A B  $3.0 \log_{10} > 3.0 \log_{10}$

\* Avian Influenza A Virus (H3N2) (Avian Ressorant) (ATCC VR-2072) A B  $> 3.5 \log_{10} > 3.5 \log_{10}$

\* Avian Influenza Virus, Type A (Turkey/WIS/66) (H9N2) A B  $> 4.5 \log_{10} > 4.5 \log_{10}$

‡ Bovine Viral Diarrhea Virus (BVDV) A B  $6.1 \log_{10} 3.8 \log_{10}$

\* Feline Calicivirus (FCV) A B  $5.79 \log_{10} > 6.06 \log_{10}$

. Hepatitis B Virus (HBV) (Duck Hepatitis B Virus-DHBV) A B  $4.5 \log_{10} 4.5 \log_{10}$

‡ Hepatitis C Virus (HCV) (Bovine Viral Diarrhea Virus-BVDV) A B  $6.1 \log_{10} 3.8 \log_{10}$

† Herpes Simplex Type 1 (Sabin) A B  $> 4.0 \log_{10} > 3.7 \log_{10}$

\* Human Coronavirus (ATCC VR-740, strain 229E) A B  $> 3.0 \log_{10} > 3.0 \log_{10}$

\* Human Immunodeficiency Virus, HTLV-III<sub>RF</sub>, strain of HIV-1

(associated with AIDS)

A B  $> 3.0 \log_{10} > 3.0 \log_{10}$

† Influenza A<sub>2</sub> (Japan 305/57) A B  $> 6.5 \log_{10} > 6.0 \log_{10}$

\* Norovirus (Norwalk Virus) (FCV) A B  $5.79 \log_{10} > 6.06 \log_{10}$

\* SARS Associated Coronavirus (ZeptoMetrix) A B  $4.03 \log_{10} 4.03 \log_{10}$

† Vaccinia (Wyeth) A B  $> 3.5 \log_{10} > 3.5 \log_{10}$



### Conclusion

Under the conditions of this investigation, Xtreme 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 1 (Sabin), **Human Coronavirus**, Human Immunodeficiency Virus (HIV-1), Influenza A<sub>2</sub> (Japan 305/57), Norovirus (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

### Results

Sample Dilution No. of Exposed Tiles No. of Tiles Showing Growth

Xtreme Detergent/Disinfectant oz/gal 10 0

Control - 10 10

### Conclusion

Under the conditions of this investigation, Xtreme 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, Xtreme 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

### Results

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, Xtreme Detergent/Disinfectant demonstrated **disinfectant** activity against *Staphylococcus aureus*, *Salmonella choleraesuis*, *Pseudomonas aeruginosa*, *Brevibacterium ammoniagenes*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, Methicillin resistant *Staphylococcus aureus* (MRSA), *Salmonella schottmuelleri*, *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 oz/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

1065

1275

99.999

99.999

99.999

65

70

185

99.999

99.999

99.999

*Escherichia coli*

(ATCC 11229)

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, cfu/ml

† % Kill calculated based on initial

inoculum control count of 75-125

x 10<sup>6</sup> cfu/ml.



### Conclusion

Under the conditions of these investigations, Xtreme 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 Xtreme 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 article Xtreme 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 Xtreme 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:** Xtreme 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  $0.3 \pm 0.1$

**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 Xtreme 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/CFR 1500.42 on  
Xtreme 11/17/07  
Herein referred to as Xtreme

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 the test article Xtreme 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 Xtreme showed no ocular reaction on all rabbits at 24 hrs 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:** Xtreme 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

Based on these results, the test article Xtreme was classified as follows:

Primary Irritation Score  $0.8 \pm 0.1$

Classification Non- Irritant

Based on the above findings, the test article Xtreme is not classified according to the US D.O.T. and the Canadian Transportation of Dangerous Goods Act.

**References:**

- (1) Buehler, E.V. 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 (1944)



Test Organisms	Dried Virus Control	Sample	Result	Log Reduction
Avian Infectious Bronchitis virus	6.42 Log <sub>10</sub>	A	<=0.5 Log <sub>10</sub>	>=5.92 Log <sub>10</sub>
Beaudette 1B42		B	<=0.5 Log <sub>10</sub>	>=5.92 Log <sub>10</sub>
	6.5 Log <sub>10</sub>	C	<=0.5 Log <sub>10</sub>	>=6.0 Log <sub>10</sub>
Avian Influenza A (H3N2) virus (Avian Reassortant) (ATCC VR-2072)		A	<=0.5 Log <sub>10</sub>	>=4.25 Log <sub>10</sub>
	4.75 Log <sub>10</sub>	B	<=0.5 Log <sub>10</sub>	>=4.25 Log <sub>10</sub>
		C	<=0.5 Log <sub>10</sub>	>=4.25 Log <sub>10</sub>
Avian Influenza A (H5N1) virus		A	<=0.5 Log <sub>10</sub>	>=6.25 Log <sub>10</sub>
	6.75 Log <sub>10</sub>	B	<=0.5 Log <sub>10</sub>	>=6.25 Log <sub>10</sub>
		A	<=0.5 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>
	4.5 Log <sub>10</sub>	B	<=0.5 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>
Canine Coronavirus ATCC VR-809		C	<=0.5 Log <sub>10</sub>	>=4.25 Log <sub>10</sub>
	4.75 Log <sub>10</sub>	A	<=0.5 Log <sub>10</sub>	>=05.75 Log <sub>10</sub>
	6.25 Log <sub>10</sub>	B	<=0.5 Log <sub>10</sub>	>=5.75 Log <sub>10</sub>
Canine Distemper virus		C	<=0.5 Log <sub>10</sub>	>=6.25 Log <sub>10</sub>
	6.75 Log <sub>10</sub>	A	<=0.5 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>
	4.5 Log <sub>10</sub>	B	<=0.5 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>
Feline Picornavirus		C	<=0.5 Log <sub>10</sub>	>=5.25 Log <sub>10</sub>
	5.75 Log <sub>10</sub>	A	<=0.27 Log <sub>10</sub>	>=4.79 Log <sub>10</sub>
	5.06 Log <sub>10</sub>	B	<=0.41 Log <sub>10</sub>	>=4.79 Log <sub>10</sub>
Hepatitis B Virus		Confirmatory B	<=0.27 Log <sub>10</sub>	>=4.79 Log <sub>10</sub>
	5.20 Log <sub>10</sub>	A	<=0.24 Log <sub>10</sub>	>=5.97 Log <sub>10</sub>
	6.21 Log <sub>10</sub>	B	<=0.42 Log <sub>10</sub>	>=5.79 Log <sub>10</sub>
Hepatitis C Virus		Confirmatory B	<=0.13 Log <sub>10</sub>	>=5.93 Log <sub>10</sub>
	6.06 Log <sub>10</sub>	A	<=0.5 Log <sub>10</sub>	>=5.0 Log <sub>10</sub>
	5.5 Log <sub>10</sub>	B	<=0.5 Log <sub>10</sub>	>=5.0 Log <sub>10</sub>
Herpes Simplex Virus Type 1		C	<=0.5 Log <sub>10</sub>	>=5.5 Log <sub>10</sub>
	6.0 Log <sub>10</sub>			

Xtreme raw material Base was evaluated in the presence of 5% serum and 400 ppm hard water with 10 minute contact time and found to be effective against the above noted viruses on hard, nonporous environmental surfaces.

Herpes Simplex Virus Type 2	6.0 Log <sub>10</sub>	A	<=0.5 Log <sub>10</sub>	>=5.5 Log <sub>10</sub>
	5.75 Log <sub>10</sub>	B	<=0.5 Log <sub>10</sub>	>=5.5 Log <sub>10</sub>
	4.5 Log <sub>10</sub>	C	<=0.5 Log <sub>10</sub>	>=5.25 Log <sub>10</sub>
Human Coronavirus	4.5 Log <sub>10</sub>	A	<=0.5 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>
		B	<=0.5 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>
	4.5 Log <sub>10</sub>	C	<=0.5 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>
Human Immunodeficiency Virus type 1 ( HIV 1)	5.75 Log <sub>10</sub>	A	<=1.5 Log <sub>10</sub>	>=4.25 Log <sub>10</sub>
		B	<=1.5 Log <sub>10</sub>	>=4.25 Log <sub>10</sub>
	4.5 Log <sub>10</sub>	C	<=1.5 Log <sub>10</sub>	>=4.25 Log <sub>10</sub>
Infectious Bovine Rhinotracheitis virus	4.5 Log <sub>10</sub>	A	<=0.0 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>
		B	<=0.0 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>
	4.75 Log <sub>10</sub>	C	<=0.0 Log <sub>10</sub>	>=4.25 Log <sub>10</sub>
Influenza A virus	6.5 Log <sub>10</sub>	A	<=0.0 Log <sub>10</sub>	>=6.0 Log <sub>10</sub>
		B	<=0.0 Log <sub>10</sub>	>=6.0 Log <sub>10</sub>
	6.0 Log <sub>10</sub>	C	<=0.0 Log <sub>10</sub>	>=5.5 Log <sub>10</sub>
Pseudorabies virus	6.25 Log <sub>10</sub>	A	<=0.5 Log <sub>10</sub>	>=5.75 Log <sub>10</sub>
		B	<=0.5 Log <sub>10</sub>	>=5.75 Log <sub>10</sub>
	5.5 Log <sub>10</sub>	C	<=0.5 Log <sub>10</sub>	>=5.0 Log <sub>10</sub>
Respiratory Syncytial virus ATCC VR-26	4.5 Log <sub>10</sub>	A	<=0.5 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>
		B	<=0.5 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>
	5.0 Log <sub>10</sub>	C	<=0.5 Log <sub>10</sub>	>=4.5 Log <sub>10</sub>
Transmissible Gastroenteritis virus	4.75 Log <sub>10</sub>	A	<=0.5 Log <sub>10</sub>	>=4.35 Log <sub>10</sub>
		B	<=0.5 Log <sub>10</sub>	>=4.25 Log <sub>10</sub>
	6.25 Log <sub>10</sub>	C	<=0.5 Log <sub>10</sub>	>=5.75 Log <sub>10</sub>
Vaccinia virus	6.75 Log <sub>10</sub>	A	<=0.5 Log <sub>10</sub>	>=6.25 Log <sub>10</sub>
		B	<=0.5 Log <sub>10</sub>	>=6.25 Log <sub>10</sub>
	6.5 Log <sub>10</sub>	C	<=0.5 Log <sub>10</sub>	>=6.0 Log <sub>10</sub>

Xtreme raw material Base was evaluated in the presence of 5% serum and 400 ppm hard water with 10 minute contact time and found to be effective against the above noted viruses on hard, nonporous environmental surfaces.



Test Organisms	Dried Virus Control	Sample	Result	Log Reduction
Canine Parvovirus Type 2b	7.5 Log <sub>10</sub>	A	<=3.5 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>
		B	<=3.5 Log <sub>10</sub>	>=4.0 Log <sub>10</sub>

Xtreme raw material Base was evaluated in the presence of 5% serum and 400 ppm hard water with 10 minute contact time and found to be effective against the above noted viruses on hard, nonporous environmental surfaces.

# Weekly Effluent Fecal Coliform Testing

Raw  
EFF  
Untreated

	Coliform Colonies Counted	x 100 /	mls of Sample Filtered	=	Colonies / 100 mls
Week:	0	x 100 /	Blank	=	0
Date:	10	x 100 /	0.10	=	10,000
Operator:	21	x 100 /	0.25	=	8,400
	49	x 100 /	0.50	=	9,800
			Average:		9,400

Raw 3 1000 mls  
EFF  
Treated  
1 mg/L

	Coliform Colonies Counted	x 100 /	mls of Sample Filtered	=	Colonies / 100 mls
Week:		x 100 /	1	=	0
Date:		x 100 /	5	=	0
Operator:		x 100 /	10	=	0
		x 100 /		=	
			Average:		0

Raw 3 1000 mls  
EFF  
Treated  
5 mg/L

	Coliform Colonies Counted	x 100 /	mls of Sample Filtered	=	Colonies / 100 mls
Week:		x 100 /	1	=	0
Date:		x 100 /	5	=	0
Operator:		x 100 /	10	=	0
		x 100 /		=	
			Average:		0

Raw 3 1000 mls  
EFF  
Treated  
10 mg/L

	Coliform Colonies Counted	x 100 /	mls of Sample Filtered	=	Colonies / 100 mls
Week:		x 100 /	1	=	0
Date:		x 100 /	5	=	0
Operator:		x 100 /	10	=	0
		x 100 /		=	
			Average:		0

Raw 3 1000 mls  
EFF  
Treated  
20 mg/L

	Coliform Colonies Counted	x 100 /	mls of Sample Filtered	=	Colonies / 100 mls
Week:		x 100 /	1	=	0
Date:		x 100 /	5	=	0
Operator:		x 100 /	10	=	0
		x 100 /		=	
			Average:		0

Report Number  
11-087-2030



13611 "B" Street • Omaha, Nebraska 68144-3693 • (402) 334-7770 • FAX (402) 334-9121  
www.midwestlabs.com

Page 1 of 2

**REPORT OF ANALYSIS**

**Mail to:** HEARTLAND SOLUTIONS INC  
STEVE ROWLEY  
626 VALLEY RIDGE CT  
GRAIN VALLEY MO 64029

For: (27016) HEARTLAND SOLUTIONS INC  
(816)867-2054

Date Reported: 03/31/11  
Date Received: 03/16/11

**ACUTE TOXICITY ANALYSIS**

Lab number: 1824580 Sample ID: XTEME MICROBIAL AGENT

Analysis	Level Found	Units	Detection Limit	Method	Analyst-Date	Verified-Date
Ammoniacal Nitrogen	n.d.	mg/L	0.10	SM 4500-NH3 C	lkd-03/21	cmw-03/28
Ammoniacal Nitrogen	n.d.	mg/L	0.10	SM 4500-NH3 C	lkd-03/21	cmw-03/28
Total chlorine	n.d.	mg/L	0.001	SM 4500-CLD	lkm-03/17	cmw-03/28
Conductance	354	uS/cm	2	SM 2510 B	jdb-03/17	cmw-03/28
Total dissolved solids	348	mg/L	10	SM 2540C	jsa-03/18	cmw-03/28
Alkalinity (Total)	83	mg CaCO3/L	10	SM 2320 B	jdb-03/21	cmw-03/28
Tua P. promelas	< 200000	NA	0.50	CALC	lkm-03/25	cmw-03/28
Tua C. dubia	714,286	NA	0.50	CALC	lkm-03/25	cmw-03/28
LC50 P. promelas	> 0.0005	%	1.00	CALC	lkm-03/25	cmw-03/28
LC50 C.dubia	0.00014	%	0.00010	CALC	lkm-03/25	cmw-03/28

**Notes:**

n.d. - Not Detected.

For questions contact

*Heather Ramig*  
Heather Ramig  
Client Service Representative  
heather@midwestlabs.com (402)829-9891

The result(s) issued on this report only reflect the analysis of the sample(s) submitted. For applicable test parameters, Midwest Laboratories is in compliance with NELAP requirements. Our reports and letters are for the exclusive and confidential use of our clients and may not be reproduced in whole or in part, nor may any reference be made to the work, the results, or the company in any advertising, news release, or other public announcements without obtaining our prior written authorization.



13611 B Street • Omaha, Nebraska 68144-3693 • (402) 334-7770 • FAX (402) 334-9121 • www.midwestlabs.com

**48 HOUR ACUTE TOXICITY TEST**  
**EPA-821-R-02-012 Non-renewal Static Serial Dilution**

Report Number:	Lab #:	Sample ID:	Sample Description:
11-087-2030	1824580	Heartland Solutions Inc.	Color: clear; Odor: slightly soapy

**STANDARD TOXICITY DATA:**

*P. promelas* Reference Toxicant: KCl LC50: 0.923 g/L Date: 03/2011

*C. dubia* Reference Toxicant: NaCl LC50: 2.249 g/L Date: 03/2011

**CONDITIONS:** Moderately Hard Synthetic Dilution Water 16hrs. Light; 8hrs. Dark

*P. promelas* Age: 2 days Time/Date Test Start: 1440 3/17/11 Time/Date End: 1510 3/19/11

*C. dubia* Age: <24 hrs Time/Date Test Start: 1440 3/17/11 Time/Date End: 1510 3/19/11

Time/Date Sample Collected: N/A Time/Date Sample Received: 1356 3/3/11

**CHEMISTRY – 5PPM EFFLUENT:**

Parameter	Result	Method
Hardness	98 mg eq CaCO <sub>3</sub> /L	Calculation
Conductivity	354 umhos/cm	EPA 120.1
Alkalinity	83 mg CaCO <sub>3</sub> /L	SM 2320B
Residual Chlorine	<0.001 mg/L	SM 4500-CLD
Dissolved Solids	348 mg/L	SM 2540C
Ammonia (pre-fish)	<0.1 mg/L	EPA 350.2
Ammonia (post-fish)	<0.1 mg/L	EPA 350.2

**RESULTS:**

Dilution	Initial		Final		# alive/20 tested			
	DO	pH	DO	pH	Fish		Fleas	
					24hr.	48hr.	24hr.	48hr.
5 ppm	7.7	8.3	7.5	8.2	20	20	0	0
4 ppm	7.9	8.2	7.5	8.2	20	20	0	0
3 ppm	8.0	8.2	7.4	8.2	20	20	0	0
2 ppm	8.0	8.2	7.3	8.3	20	20	0	0
1 ppm	8.0	8.2	7.4	8.3	20	20	20	17
Control	8.0	8.2	7.5	8.3	20	20	20	20

**FATHEAD MINNOW (*P. promelas*)**

LC50: >.0005%

TUa: <200,000

Method: N/A

**WATER FLEA (*C. dubia*)**

LC50: .00014%

TUa: 714,286

Method: Two-Point Interpolation

Analyst

Laura Rivas

Date 3/28/11

Second Party Reviewer

Cl Vanenburg

Date 3/28/11

Client Services Representative

Heather Kamig

Date 3/28/11



**PDC Laboratories, Inc.**

3278 N. Highway 67 - Florissant, MO 63033  
(314) 432-0550 - (800) 333-FAST (3278) - FAX (314) 432-4977



Stover, City of  
PO Box 370  
Stover, MO 65078  
Attn: Scott Beckmann

Date Received: 08/31/11 10:55  
Report Date: 09/19/11  
Customer #: 277228

Sample No: 1091895-01  
Sample Description: Effluent

Collect Date: 08/30/2011 14:20  
Matrix: Waste water

Parameters	Result	Qual	Analysis Date	Analyst	Method
------------	--------	------	---------------	---------	--------

WET test was subcontracted out. See attachment.





**PDC Laboratories, Inc.**

3278 N. Highway 67 - Florissant, MO 63033  
(314) 432-0550 - (800) 333-FAST (3278) - FAX (314) 432-4977



Stover, City of  
PO Box 370  
Stover, MO 65078  
Attn: Scott Beckmann

Date Received: 08/31/11 10:55  
Report Date: 09/19/11  
Customer #: 277228

---

**Notes**

---

This report shall not be reproduced, except in full, without the written approval of the laboratory.

PDC Laboratories participates in the following accreditation/certification and proficiency programs at the following locations.  
Endorsement by Federal or State Governments or their agencies is not implied.

PIA PDC Laboratories - Peoria, IL

NELAC Accreditation for Drinking Water, Wastewater, Hazardous and Solid Wastes Fields of Testing through IL EPA Lab No. 100230

Illinois Department of Public Health Bacteriological Analysis in Drinking Water Approved Laboratory Registry No. 17553

Drinking Water Certifications: Kansas (E-10338); Missouri (870); Wisconsin (998284430); Indiana (C-IL-040); Iowa (240)

Wastewater Certifications: Arkansas (88-0677); Wisconsin (998284430); Iowa (240); Kansas (E-10335)

Hazardous/Solid Waste Certifications: Arkansas (88-0677); Wisconsin (998284430); Iowa (240); Kansas (E-10335)

UST Certification; Iowa (240)

SPM PDC Laboratories - Springfield, MO

EPA DMR-QA Program

STL PDC Laboratories - St. Louis, MO

NELAC Accreditation for Wastewater, Hazardous and Solid Wastes Fields of Testing through KS EPA Lab No. E-10389

*Barbara G Pandolfo*

---

Certified by: Barb Pandolfo, Project Manager

# Environmental Analysis South, Inc.

4000 East Jackson Blvd. • Jackson, MO 63755 • 573-204-8817 • Fax 573-204-8818



## REPORT OF ACUTE TOXICITY TESTING

Stover Southwest Lagoon

Outfall 001 (24 hr composite), AEC=100%

MO-0047058

EAS LOG# 1316318

August 31, 2011 through September 2, 2011

### Tests performed by:

John P. Clippard / Chemical Analyst at Environmental Analysis South (EAS)

Kelly J. Ray / Biologist at Environmental Analysis South (EAS)

Sara C. Shields / Lab Supervisor - Chemist at Environmental Analysis South (EAS)

David F. Warren / Lab Director - Chemist at Environmental Analysis South (EAS)

1. Report Summation
  - 1.1. Data Summation
  - 1.2. Conclusion
2. Method Summation
  - 2.1. Test Conditions and Methods
  - 2.2. Potassium chloride Reference Salt Test
    - 2.2.1. *Pimephales promelas* data
    - 2.2.2. *Ceriodaphnia dubia* data
  - 2.3. Literature Cited
3. Raw Data Bench Sheets
  - 3.1. Initial observations (page 1)
  - 3.2. Zero hour Observations (page 1)
  - 3.3. Twenty-four (24) hour Observations (page 1)
  - 3.4. Forty-eight (48) hour Observations (page 1)
  - 3.5. Survival Data Table (page 2)
  - 3.6. Test Comments (page 3)
4. Chain of Custody
5. MO DNR "Whole Effluent Toxicity (WET) Test Report (Form 780-1899)

# Environmental Analysis South, Inc.

4000 East Jackson Blvd. • Jackson, MO 63755 • 573-204-8817 • Fax 573-204-8818



**REPORT OF ACUTE TOXICITY TESTING**  
Stover Southwest Lagoon  
Outfall 001 (24 hr composite), AEC=100%  
MO-0047058  
EAS LOG# 1316318  
August 31, 2011 through September 2, 2011

## 1. REPORT SUMMATION:

### 1.1. Single Dilution Data Summation

	<i>Pimephales promelas</i> Acute Toxicity Test	<i>Ceriodaphnia dubia</i> Acute Toxicity Test
Survival in the Effluent at 48 Hours	100%	100%
Survival in the Reconstituted Control (RC) at 48 Hours	100%	100%
Survival in the Upstream Control (UC) at 48 Hours	100%	100%
Statistical Results Comparing the Survival Data of the Effluent with the Control (arc sine square root transformation)	No Significant Difference at alpha = 0.05  <b>PASS</b>	No Significant Difference at alpha = 0.05  <b>PASS</b>

\* Indicates a significant difference at alpha = 0.5 between effluent and control survival data.

**Conclusion:** The mortality observed with both species was determined not to be significantly different than that observed in the control sample. Based on these results the outfall passed the whole effluent toxicity test with both indicator species.

Approved by \_\_\_\_\_

Sara C. Shields, Chemist

# Environmental Analysis South, Inc.

4000 East Jackson Blvd. • Jackson, MO 63755 • 573-204-8817 • Fax 573-204-8818



**REPORT OF ACUTE TOXICITY TESTING**  
**Stover Southwest Lagoon**  
**Outfall 001 (24 hr composite), AEC=100%**  
**MO-0047058**  
**EAS LOG# 1316318**  
**August 31, 2011 through September 2, 2011**

## 2. TEST METHOD SUMMARY

### 2.1. TEST CONDITIONS AND METHODS:

	<i>Ceriodaphnia dubia:</i>	<i>Pimephales promelas:</i>
Test duration:	48 hours	48 hours
Temperature:	24 - 26 degree Celsius	24 - 26 degree Celsius
Light quality:	Ambient laboratory illumination	Ambient laboratory illumination
Photoperiod:	16 hour light, 8 hours dark	16 hour light, 8 hours dark
Control Water:	Moderately Hard Reconstituted Water	Moderately Hard Reconstituted Water
Dilution Water:	Upstream Water - If unavailable or toxic, then control water will be used.	Upstream Water - If unavailable or toxic, then control water will be used.
Size of test vessel:	30 milliliters	250 milliliters
Volume of test solution:	15 milliliters	200 milliliters
Age of test organisms:	<24 hours	1 -14 days (all same age)
Number of organisms/test vessel:	5	10
Number of replicates/concentration:	4	4
Number of organisms/concentration:	20	40 for a single dilution test and 20 for a multiple dilution test
Feeding regime:	None (fed prior to test)	None (fed prior to test)
Aeration:	None	None
Test acceptability criterion:	90% or greater survival in controls	90% or greater survival in controls

The methodology used for the chemistry data was taken from the *Standard Methods for the Examination of Water and Wastewater*, 18<sup>th</sup> edition (1992). The exception was hardness, which was determined using a Hach EDTA titration test kit. The toxicity tests follow guidelines laid out in the permittee's NPDES permit and were conducted according to EPA approved methods (USEPA 2002).

All test organisms were cultured according to EPA approved methods (USEPA 2002). The *Ceriodaphnia dubia* and the *Pimephales promelas* were obtained from C-K Associates Inc. located in Baton Rouge, Louisiana and shipped overnight for use in the whole effluent toxicity test.

# Environmental Analysis South, Inc.

4000 East Jackson Blvd. • Jackson, MO 63755 • 573-204-8817 • Fax 573-204-8818



## REPORT OF ACUTE TOXICITY TESTING

Stover Southwest Lagoon

Outfall 001 (24 hr composite), AEC=100%

MO-0047058

EAS LOG# 1316318

August 31, 2011 through September 2, 2011

### 2.2. REFERENCE TOXICITY TEST:

Environmental Analysis South performs monthly reference toxicity tests. The most recent reference test was initiated on August 3, 2011 using KCL Lot #41713. Following are the results:

2.2.1. *P. promelas* - 48 hr. Acute Test –  $LC_{50}$  = 1.049 g/l 95%CI (0.705-1.392 g/l)

EAS %CV = 16.4%

National Warning Limits (75<sup>th</sup> percentile) = 19%CV

National Control Limits (90<sup>th</sup> percentile) = 33%CV

2.2.2. *C. dubia* - 48 hr. Acute Test –  $LC_{50}$  = 0.451 g/l 95%CI (0.295-0.607g/l)

EAS %CV = 17.3%

National Warning Limits (75<sup>th</sup> percentile) = 29%CV

National Control Limits (90<sup>th</sup> percentile) = 34%CV

### 2.3. LITERATURE CITED:

1. APHA. 1992. *Standard methods for the examination of water and wastewater*, 18th Ed. American Public Health Association, Washington, D.C
2. USEPA. 2002. *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms*, 5th Ed. EPA-821-R-02-012
3. USEPA 2000. *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications under the National Pollutant Discharge Elimination System, (Table B-2)*. June 2000. EPA 833-R-00-003.

WHOLE EFFLUENT TEST conducted in accordance with US EPA 600/4-90/027  
Fifth Edition October 2002

CLIENT NAME:		Stover Southwest Lagoon, Outfall 001, 24 hr composite									
NPDES NUMBER:		MO-0047058									
TYPE OF METHOD:		single dilution, 48 hrs, PP & CD, AEC=100%									
DATE & TIME OF COLLECTION:		08/30/11 1420 hrs by Steve Holloway									
DATE & TIME OF SUBMISSION:		08/30/11 1055 hrs by UPS									
INITIAL OBSERVATIONS		DATE	TIME	ANALYST	QC LOT	QC EXP VALUE	INT EFFL	INT UC	INT RC	Upstream: Gabriel Creek	
LOG NUMBER / ID NUMBER							1316318	1316318A	RC4020	Collected: 08/30/11 1440 hrs by S.H.	
TEMPERATURE °C RECEIVED		08/31/11	1100 hrs	SCS	SB114 (8-8-9-2)	8.88	7.53	7.60	7.42		
SPECIFIC CONDUCTANCE umhos		08/31/11	1100 hrs	SCS	EAS 106		6	6	25		
HARDNESS - ppm		08/31/11	1100 hrs	SCS	ERA506-010511(401-457)	439	518	519	248		
CHLORINE - ppm		08/31/11	1100 hrs	SCS	ERA P170-507(107-134)	120	200	160	80		
DISSOLVED OXYGEN - ppm		08/31/11	1100 hrs	SCS	tap water	+	<0.04	<0.04	<0.04		
TOTAL ALKALINITY - ppm		08/31/11	1100 hrs	SCS	cal@840		4.8	4.6	7		
INITIAL AMMONIA - ppm		08/31/11	1600 hrs	SCS	ERA506-010511(60.1-71.9)	68.8	194	188	64.3		
TOTAL DISSOLVED SOLIDS - ppm		09/02/11	1018 hrs	JPC	EAS #1981 (8-12)	10	0.485	0.192	<0.05		
0 HOUR OBSERVATIONS		DATE	TIME	ANALYST	QC LOT	QC EXP VALUE	RC	UC	100%	50%	25%
pH - SU		08/31/11	1200 hrs	SCS	SB114 (8-8-9-2)	8.88	7.48	7.83	7.68	12.50%	6.25%
TEMPERATURE °C		08/31/11	1200 hrs	SCS	EAS 106		24.1	23.8	24.0		
SPECIFIC CONDUCTANCE umhos		08/31/11	1200 hrs	SCS	ERA506-010511(401-457)	439	245	475	518		
DISSOLVED OXYGEN - ppm		08/31/11	1200 hrs	SCS	cal@840		6.8	8.3	8.4		
24 HOUR OBSERVATIONS - PP		DATE	TIME	ANALYST	QC LOT	QC EXP VALUE	RC	UC	100%	50%	25%
pH - SU		09/01/11	1200 hrs	SCS	SB114 (8-8-9-2)	8.91	7.14	7.96	7.88	12.50%	6.25%
TEMPERATURE °C		09/01/11	1200 hrs	SCS	EAS 106		24.9	24.9	24.9		
SPECIFIC CONDUCTANCE umhos		09/01/11	1200 hrs	SCS	ERA506-010511(401-457)	431	257	494	528		
DISSOLVED OXYGEN - ppm		09/01/11	1200 hrs	SCS	cal@840		6.3	5.7	5		
48 HOUR OBSERVATIONS - PP		DATE	TIME	ANALYST	QC LOT	QC EXP VALUE	RC	UC	100%	50%	25%
pH - SU		09/02/11	1200 hrs	SCS	SB114 (8-8-9-2)	8.93	7.53	8.14	8.09	12.50%	6.25%
TEMPERATURE °C		09/02/11	1200 hrs	SCS	EAS 106		24.4	24.4	24.4		
SPECIFIC CONDUCTANCE umhos		09/02/11	1200 hrs	SCS	ERA506-010511(401-457)	493	279	518	544		
DISSOLVED OXYGEN - ppm		09/02/11	1200 hrs	SCS	cal@840		6.3	5.8	5.6		
FINAL AMMONIA - ppm					EAS #2375 (8-12)						
24 HOUR OBSERVATIONS - CD		DATE	TIME	ANALYST	QC LOT	QC EXP VALUE	RC	UC	100%	50%	25%
pH - SU		09/01/11	1200 hrs	SCS	SB114 (8-8-9-2)	8.91	8.35	8.13	8.21	12.50%	6.25%
TEMPERATURE °C		09/01/11	1200 hrs	SCS	EAS 106		24.9	24.9	24.9		
SPECIFIC CONDUCTANCE umhos		09/01/11	1200 hrs	SCS	ERA506-010511(401-457)	431	245	470	514		
DISSOLVED OXYGEN - ppm		09/01/11	1200 hrs	SCS	cal@840		6.4	6.1	6.2	12.50%	6.25%
48 HOUR OBSERVATIONS - CD		DATE	TIME	ANALYST	QC LOT	QC EXP VALUE	RC	UC	100%	50%	25%
pH - SU		09/02/11	1200 hrs	SCS	SB114 (8-8-9-2)	8.93	8.47	8.36	8.41		
TEMPERATURE °C		09/02/11	1200 hrs	SCS	EAS 106		24.4	24.4	24.4		
SPECIFIC CONDUCTANCE umhos		09/02/11	1200 hrs	SCS	ERA506-010511(401-457)	493	279	492	500		
DISSOLVED OXYGEN - ppm		09/02/11	1200 hrs	SCS	cal@840		6.2	6.1	5.9		
FINAL AMMONIA - ppm					EAS #2375 (8-12)						

Approved by: *[Signature]* Date: 09/08/2011

WHOLE EFFLUENT TEST conducted in accordance with US EPA 600/4-90/027  
Fifth Edition October 2002

Stover Southwest Lagoon, Outfall 001, 24 hr composite EAS LOG# 1316318

Date Test Began: August 31, 2011 Time Test Began: 1200 hrs Analyst 1: DFW  
Date Test Finished: September 2, 2011 Time Test Finished: 1200 hrs Analyst 2: KJR  
Analyst 3: SCS

*P. promelas* (PP) AGE: 6 days HATCH NUMBER: 8112 c-k

PERIOD	RC	UC	100%	50%	25%	12.50%	6.25%	X% AEC
0 HR-PP	10,10,10,10	10,10,10,10	ALIVE	ALIVE	ALIVE	ALIVE	ALIVE	ALIVE
24 HR-PP	10,10,10,10	10,10,10,10	10,10,10,10					
48 HR-PP	10,10,10,10	10,10,10,10	10,10,10,10					

*Coriodaphnia dubia* (CD) AGE: <24 hours HATCH NUMBER: 2373 c-k

PERIOD	RC	UC	100%	50%	25%	12.50%	6.25%	X% AEC
0 HR-CD	5,5,5,5	5,5,5,5	ALIVE	ALIVE	ALIVE	ALIVE	ALIVE	ALIVE
24 HR-CD	5,5,5,5	5,5,5,5	5,5,5,5					
48 HR-CD	5,5,5,5	5,5,5,5	5,5,5,5					

Approved by: *[Signature]*

Date: 09/08/2011





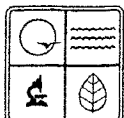
ALL SHADED AREAS MUST BE COMPLETED BY CLIENT (PLEASE PRINT)

1 CLIENT INFORMATION		2 PROJECT INFORMATION		3 MEANS SHIPPED		4 (FOR LAB USE ONLY)	
PHONE NUMBER	P.O. NUMBER	PHONE NUMBER	FAX NUMBER	EMAIL ADDRESS	LOGGED BY:	LAB PROJ. #	TEMPLATE:
<b>CLIENT:</b> City of St. Louis <b>PROJECT:</b>		<b>PROJECT NUMBER:</b> <b>PHONE NUMBER:</b>		<b>MEANS SHIPPED:</b> <b>EMAIL ADDRESS:</b>		<b>LOGGED BY:</b> <b>LAB PROJ. #</b> <b>TEMPLATE:</b> <b>PROJ. MGR.:</b>	
<b>2 SAMPLE DESCRIPTION</b> AS YOU WANT ON REPORT		<b>DATE COLLECTED</b> <b>TIME COLLECTED</b>		<b>MATRIX TYPE:</b> WW-WASTEWATER DW-DRINKING WATER GW-GROUND WATER WWSL-SLUDGE NAS-SOLID LCH-LEACHATE WSL-WASTEWATER SOL-SOLIDS		<b>REMARKS</b> temp rec id = 60°C	
<b>Sample Description:</b> Effluent		<b>Date Collected:</b> 8/30/11 <b>Time Collected:</b> 14:20		<b>Matrix Type:</b> X		<b>Remarks:</b> temp rec id = 60°C	
<b>Sample Description:</b> Downstream		<b>Date Collected:</b> 8/30/11 <b>Time Collected:</b> 14:40		<b>Matrix Type:</b> X		<b>Remarks:</b> temp rec id = 60°C	

**5 TURNAROUND TIME** (RUSH TAT IS SUBJECT TO PDC LABS APPROVAL AND SURCHARGE)  
 NORMAL (6-10 Bus. Days) RUSH (5 Bus. Days) *Fastrack* (3 Bus. Days) 1-2 Bus. Days Same Day  
 DATE DUE \_\_\_\_\_

**6 RESULTS BY:** E-MAIL FAX PHONE CALL PHONE/FAX# IF DIFFERENT FROM ABOVE

7 RECEIVED BY:		8 COMMENTS (FOR LAB USE ONLY)	
TIME	DATE	TIME	DATE
15:40	8/30/11	15:40	8/30/11
16:40	8/30/11	10:55	8/31/11
<b>RELINQUISHED BY: (SIGNATURE)</b> <i>Batolyn Mayes</i>		<b>CHILL PROCESS STARTED PRIOR TO RECEIPT</b> SAMPLE(S) RECEIVED ON ICE PROPER BOTTLES RECEIVED IN GOOD CONDITION BOTTLES FILLED WITH ADEQUATE VOLUME SAMPLES RECEIVED WITHIN HOLD TIME(S) (EXCLUDES TYPICAL FIELD PARAMETERS) DATE AND TIME TAKEN FROM SAMPLE BOTTOM	
<b>RELINQUISHED BY: (SIGNATURE)</b> <i>Batolyn Mayes</i>		<b>DATE AND TIME TAKEN FROM SAMPLE BOTTOM</b> DATE AND TIME TAKEN FROM SAMPLE BOTTOM	



MISSOURI DEPARTMENT OF NATURAL RESOURCES  
WATER PROTECTION PROGRAM - P.O. BOX 176, JEFFERSON CITY MO, 65102  
**WHOLE EFFLUENT TOXICITY (WET) TEST REPORT**  
(TO BE ATTACHED TO WET TESTS FOR SUBMISSION TO THE REGULATORY AUTHORITY)

**PART A - TO BE COMPLETED IN FULL BY PERMITTEE**

FACILITY NAME Stover Southwest Lagoon		DATE & TIME COLLECTED EFFLUENT 08/30/11 1420 UPSTREAM 08/30/11 1440	
PERMIT NUMBER MO-0047058		PERMIT OUTFALL NUMBER Outfall # 001	
COLLECTOR'S NAME Steve Holloway			
RECEIVING STREAM COLLECTION SITE AND DESCRIPTION Gabriel Creek			
PERMIT ALLOWABLE EFFLUENT CONCENTRATION (AEC) 100%		EFFLUENT SAMPLE TYPE (CHECK ONE) <input checked="" type="checkbox"/> 24HR COMPOSITE <input type="checkbox"/> GRAB <input type="checkbox"/> OTHER	
SAMPLE NUMBER EFFLUENT 1316318 UPSTREAM 1316318A		UPSTREAM SAMPLE TYPE (CHECK ONE) <input type="checkbox"/> 24HR COMPOSITE <input checked="" type="checkbox"/> GRAB <input type="checkbox"/> OTHER	
PERMITTED EFFLUENT DAILY MAXIMUM LIMITATION FOR CHLORINE mg/L		PERMITTED EFFLUENT DAILY MAXIMUM LIMITATION FOR AMMONIA mg/L	

**PART B - TO BE COMPLETED IN FULL BY PERFORMING LABORATORY**

PERFORMING LABORATORY Environmental Analysis South, Inc.		TEST TYPE Acute Static Non renewal Test Single Dilution	
FINAL REPORT NUMBER MO_1316318		TEST DURATION 48 hour	
DATE OF LAST REFERENCE TOXICANT TESTING August 3, 2011		TEST METHOD Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms	
DATE AND TIME SAMPLES RECEIVED AT LABORATORY 08/31/11 1055 hrs by UPS		TEST START DATE AND TIME 08/31/11 1200 hrs	TEST END DATE AND TIME 09/02/11 1200 hrs
SAMPLE DECHLORINATED PRIOR TO ANALYSIS? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO EFFLUENT UPSTREAM		TEST ORGANISM #1 AND AGE Pimephales promelas 6 days	TEST ORGANISM #2 AND AGE Ceriodaphnia dubia < 24 hours
SAMPLE FILTERED <sup>1</sup> PRIOR TO ANALYSIS? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO EFFLUENT UPSTREAM		90% OR GREATER SURVIVAL IN SYNTHETIC CONTROL? <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	DILUTION WATER USED TO ACHIEVE AEC none
FILTER MESH SIEVE SIZE <sup>2</sup> None		EFFLUENT ORGANISM #1 % MORTALITY AT AEC 0 %	EFFLUENT ORGANISM #2 % MORTALITY AT AEC 0 %
SAMPLE AERATED DURING TESTING? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO		UPSTREAM ORGANISM #1 % MORTALITY 0 %	UPSTREAM ORGANISM #2 % MORTALITY 0 %
pH ADJUSTED? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO EFFLUENT UPSTREAM		TEST RESULT AT AEC FOR ORGANISM #1 <input checked="" type="checkbox"/> PASS <input type="checkbox"/> FAIL	TEST RESULT AT AEC FOR ORGANISM #2 <input checked="" type="checkbox"/> PASS <input type="checkbox"/> FAIL

**MINIMUM REQUIRED ANALYTICAL RESULTS FOR THE 100% EFFLUENT SAMPLE**

PARAMETER	RESULT	METHOD	WHEN ANALYZED
Temperature °C	6	SM18 2550B stored at 4 degree C until test setup	08/31/11 1100 hrs
pH Standard Units	7.53	SM18 4500-H B	08/31/11 1100 hrs
Conductance µMohs	518	SM18 2510B	08/31/11 1100 hrs
Dissolved Oxygen mg/L	4.8	SM18 4500-O G	08/31/11 1100 hrs
Total Residual Chlorine mg/L	<0.04	SM18 4500-CI G	08/31/11 1100 hrs
Unionized Ammonia mg/L	0.485x0.02=0.010	SM18 4500-NH3 F @ 25 degree C	09/02/11 1018 hrs
*Total Alkalinity mg/L	194	SM18 2320B	08/31/11 1600 hrs
*Total Hardness mg/L	200	SM18 2340 C	08/31/11 1100 hrs

\*Recommended by USEPA guidance, not a required analysis.

<sup>1</sup> Samples shall only be filtered if indigenous organisms are present that may be confused with, or attack, the test organisms.

<sup>2</sup> Filters shall have a sieve size of 60 microns or greater.

## WHOLE EFFLUENT TOXICITY (WET) TEST REPORT

(TO BE ATTACHED TO WET TESTS FOR SUBMISSION TO THE REGULATORY AUTHORITY)

### MINIMUM REQUIRED ANALYTICAL RESULTS FOR THE 100% UPSTREAM SAMPLE<sup>3</sup>

PARAMETER	RESULT	METHOD	WHEN ANALYZED
Temperature °C	6	SM18 2550B stored at 4 degree C until test setup	08/31/11 1100 hrs
pH Standard Units	7.60	SM18 4500-H B	08/31/11 1100 hrs
Conductance µMols	519	SM18 2510B	08/31/11 1100 hrs
Dissolved Oxygen mg/L	4.6	SM18 4500-O G	08/31/11 1100 hrs
Total Residual Chlorine mg/L	<0.04	SM18 4500-Cl G	08/31/11 1100 hrs
Unionized Ammonia mg/L	0.192x0.02<0.010	SM18 4500-NH3 F @ 25 degree C	09/02/11 1018 hrs
*Total Alkalinity mg/L	188	SM18 2320B	08/31/11 1600 hrs
*Total Hardness mg/L	160	SM18 2340 C	08/31/11 1100 hrs

\*Recommended by USEPA guidance, not a required analysis.

### PRELIMINARY TEST ACCEPTABILITY MATRIX (FOR USE BY PERMITTEE IN DETERMINING TEST VALIDITY)

**PERMIT ALLOWABLE EFFLUENT CONCENTRATION (AEC):** As indicated on permit. Test is invalid otherwise.

**EFFLUENT SAMPLE TYPE:** As indicated on permit. Test is invalid otherwise.

**TEST TYPE:** Acute Static Non-Renewal Test or other as indicated on permit. Test is invalid otherwise.

**TEST DURATION:** Forty-eight (48) hours or as indicated on permit. Test is invalid otherwise.

**TEST ORGANISMS:** As indicated on permit. Test is invalid otherwise.

**DILUTION WATER USED TO ACHIEVE AEC:** Upstream receiving water required if available.

**TEST METHOD:** The only acceptable method is the *most current edition* of Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, or other as specifically assigned by EPA for determining NPDES compliance. Test is invalid otherwise.

**TEST START DATE & TIME:** Unless otherwise specified in writing by EPA, if >36 hours lapse between collection and initiation, test is invalid.

**FILTER MESH SIEVE SIZE:** Unless otherwise specified in writing by EPA, if sieve size is smaller than 60 microns, test is invalid.

**90% OR GREATER SURVIVAL IN LABORATORY CONTROL(S) (Y/N):** If NO, test is invalid.

PARAMETER	RESULT	NOTES	WHEN ANALYZED
Temperature °C	0 - 6	Unless received by the laboratory on the same day as collected, values outside this range invalidate the test.	Upon receipt

<sup>3</sup> Where no upstream control is available, enter results from laboratory or synthetic control.

Report Number  
12-045-2066



Page 1 of 4

13611 "B" Street • Omaha, Nebraska 68144-3693 • (402) 334-7770 • FAX (402) 334-9121  
www.midwestlabs.com

**REPORT OF ANALYSIS**

**Mail to:** HEARTLAND SOLUTIONS INC  
STEVE ROWLEY  
626 VALLEY RIDGE CT  
GRAIN VALLEY MO 64029

For: (27016) HEARTLAND SOLUTIONS INC  
(816)867-2054

Date Reported: 02/16/12  
Date Received: 02/02/12  
Date Sampled: Not Supplied  
Time Sampled: Not Supplied

**ACUTE TOXICITY ANALYSIS**

Lab number: 1950753 Sample ID: **EXTREME** MIC-FORMULA XM 100

Analysis	Level Found	Units	Detection Limit	Method	Analyst-Date	Verified-Date
Ammoniacal Nitrogen	n.d.	mg/L	0.10	SM 4500-NH3 C	lkd-02/09	cmw-02/14
Ammoniacal Nitrogen	n.d.	mg/L	0.10	SM 4500-NH3 C	lkd-02/09	cmw-02/14
Total chlorine	n.d.	mg/L	0.001	SM 4500-CLD	lkm-02/07	cmw-02/14
Conductance	342	uS/cm	2	SM 2510 B	jdb-02/07	cmw-02/14
Total dissolved solids	208	mg/L	10	SM 2540C	jsa-02/08	cmw-02/14
Alkalinity (Total)	84	mg CaCO3/L	10	SM 2320 B	jdb-02/08	cmw-02/14
Tua P. promelas	< 100000	NA	0.50	CALC	lkm-02/13	cmw-02/14
Tua C. dubia	434,783	NA	0.50	CALC	lkm-02/13	cmw-02/14
LC50 P. promelas	> .001	%	1.00	CALC	lkm-02/13	cmw-02/14
LC50 C.dubia	n.d.	%	1.00	CALC	lkm-02/13	cmw-02/14

**Notes:**

n.d. - Not Detected.

For questions contact

  
Heather Ramig  
Client Service Representative  
heather@midwestlabs.com (402)829-9891

The result(s) issued on this report only reflect the analysis of the sample(s) submitted. For applicable test parameters, Midwest Laboratories is in compliance with NELAC requirements.  
Our reports and letters are for the exclusive and confidential use of our clients and may not be reproduced in whole or in part, nor may any reference be made to the work, the results, or the company in any advertising, news release, or other public announcements without obtaining our prior written authorization.



13611 B Street • Omaha, Nebraska 68144-3693 • (402) 334-7770 • FAX (402) 334-9121 • www.midwestlabs.com  
Report Number 12-045-2066

Page 2 of 4

**48 HOUR ACUTE TOXICITY TEST**  
EPA-821-R-02-012 Non-renewal Static Serial Dilution

<b>Report Number:</b> 12-045-2066	<b>Lab #:</b> 1950753	<b>Sample ID:</b> Heartland Solutions Inc. - H <sub>2</sub> O Resolution	<b>Sample Description:</b> Color: clear; Odor: slightly soapy
--------------------------------------	--------------------------	--	--

**STANDARD TOXICITY DATA:**

*P. promelas* Reference Toxicant: KCl LC50: 0.961 g/L Date: 01/2012

*C. dubia* Reference Toxicant: NaCl LC50: 1.679 g/L Date: 01/2012

**CONDITIONS:** 25°C±1°C Moderately Hard Synthetic Dilution Water 16hrs. Light: 8hrs. Dark @ 50-100 ft-c

*P. promelas* Age: 2 days Time/Date Test Start: 1230 2/7/12 Time/Date End: 1140 2/9/12

*C. dubia* Age: <24 hrs Time/Date Test Start: 1230 2/7/12 Time/Date End: 1140 2/9/12

Time/Date Sample Prepared: 12/15/08

Time/Date Sample Received: 1228 2/2/12

**CHEMISTRY – 10PPM EFFLUENT:**

Parameter	Result	Method
Hardness	103 mg eq CaCO <sub>3</sub> /L	Calculation
Conductivity	342 umhos/cm	EPA 120.1
Alkalinity	84 mg CaCO <sub>3</sub> /L	SM 2320B
Residual Chlorine	<0.001 mg/L	SM 4500-CLD
Dissolved Solids	208 mg/L	SM 2540C
Ammonia (pre-fish)	<0.1 mg/L	EPA 350.2
Ammonia (post-fish)	<0.1 mg/L	EPA 350.2

**RESULTS:**

Dilution	Initial		Final		# alive/20 tested			
	DO	pH	DO	pH	Fish		Fleas	
					24hr.	48hr.	24hr.	48hr.
10 ppm	8.6	8.2	7.8	8.1	20	20	0	0
7 ppm	8.6	8.2	7.4	8.2	20	20	0	0
5 ppm	8.6	8.2	7.8	8.2	20	20	0	0
3 ppm	8.6	8.2	7.3	8.2	20	20	10	7
Control	8.7	8.3	7.7	8.2	20	20	20	20

**FATHEAD MINNOW (*P. promelas*)**

LC50: >.001%

TUa: <100,000

Method: N/A

**WATER FLEA (*C. dubia*)**

LC50: .00023%

TUa: 434,783

Method: Two-Point Interpolation

Analyst

Lauren Rivers

Date 2/14/12

Second Party Reviewer

Chloe Burgess

Date 2/14/12

Client Services Representative

Heather Ramirez

Date 2/14/12





13611 B Street • Omaha, Nebraska 68144-3693 • (402) 334-7770 • FAX (402) 334-9121 • www.midwestlabs.com  
Report Number 12-045-2066 Page 3 of 4

**48 HOUR ACUTE TOXICITY TEST**  
EPA-821-R-02-012 Non-renewal Static Serial Dilution

<b>Report Number:</b> 12-045-2066	<b>Lab #:</b> 1950753	<b>Sample ID:</b> Heartland Solutions Inc. - Xtreme Microbial Agent	<b>Sample Description:</b> Color: clear; Odor: slightly soapy
--------------------------------------	--------------------------	---	--

**STANDARD TOXICITY DATA:**

*P. promelas* Reference Toxicant: KCl LC50: 0.961 g/L Date: 01/2012

*C. dubia* Reference Toxicant: NaCl LC50: 1.679 g/L Date: 01/2012

**CONDITIONS:** 25°C±1°C Moderately Hard Synthetic Dilution Water 16hrs. Light; 8hrs. Dark @ 50-100 ft-c

*P. promelas* Age: 2 days Time/Date Test Start: 1230 2/7/12 Time/Date End: 1140 2/9/12

*C. dubia* Age: <24 hrs Time/Date Test Start: 1230 2/7/12 Time/Date End: 1140 2/9/12

Time/Date Sample Prepared: 12/15/08

Time/Date Sample Received: 1228 2/2/12

**CHEMISTRY – 10PPM EFFLUENT:**

Parameter	Result	Method
Hardness	103 mg eq CaCO <sub>3</sub> /L	Calculation
Conductivity	342 umhos/cm	EPA 120.1
Alkalinity	84 mg CaCO <sub>3</sub> /L	SM 2320B
Residual Chlorine	<0.001 mg/L	SM 4500-CLD
Dissolved Solids	208 mg/L	SM 2540C
Ammonia (pre-fish)	<0.1 mg/L	EPA 350.2
Ammonia (post-fish)	<0.1 mg/L	EPA 350.2

**RESULTS:**

Dilution	Initial		Final		# alive/20 tested			
	DO	pH	DO	pH	Fish		Fleas	
					24hr.	48hr.	24hr.	48hr.
10 ppm	8.6	8.2	7.8	8.1	20	20	0	0
7 ppm	8.6	8.2	7.4	8.2	20	20	0	0
5 ppm	8.6	8.2	7.8	8.2	20	20	0	0
3 ppm	8.6	8.2	7.3	8.2	20	20	10	7
Control	8.7	8.3	7.7	8.2	20	20	20	20

**FATHEAD MINNOW (*P. promelas*)**

LC50: >.001%

TUa: <100,000

Method: N/A

**WATER FLEA (*C. dubia*)**

LC50: .00023%

TUa: 434,783

Method: Two-Point Interpolation

Analyst

Lauren P. Wiers

Date 2/14/12

Second Party Reviewer

Chloe A. Wiers

Date 2/14/12

Client Services Representative

Heather K. Kramig

Date 2/14/12

# CETIS Analysis Detail

Report Number  
12-045-2006

Linear Interpolation: Page 1 of 1  
Report Date: 13 Feb-12 10:24 AM  
Analysis: 15-7269-4492/1950753cd

Page 4 of 4  
Midwest Laboratories, Inc.

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
48h Proportion Survived	Linear Interpolation	09-6913-1489	09-6913-1489	13 Feb-12 10:24 AM	CETISv1.1.2

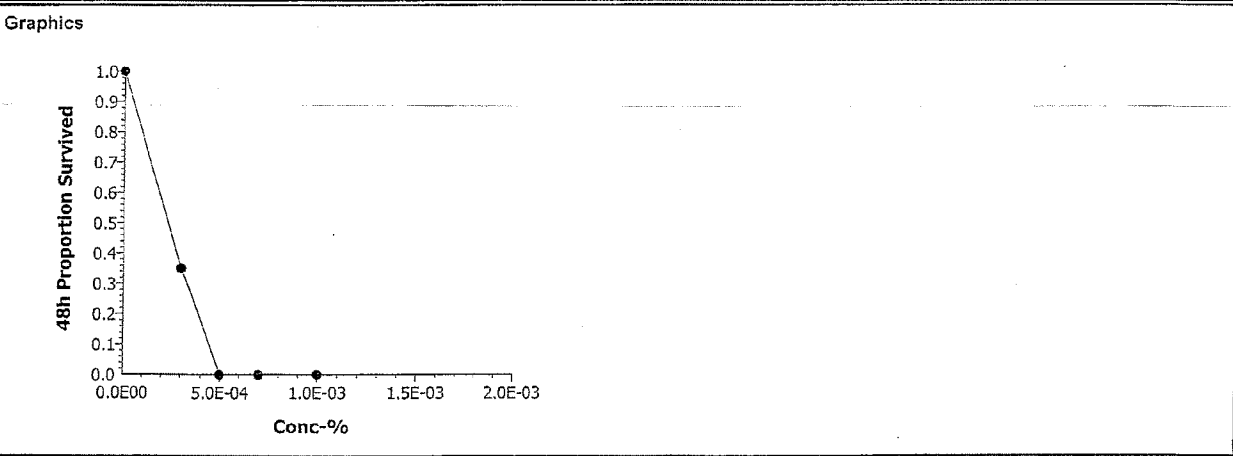
Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	57951	200	Yes	Two-Point Interpolation

Test Acceptability				
Attribute	Statistic	TAC Range	Overlap	Decision
Control Response	1	0.9 - NL	Yes	Passes acceptability criteria

Point Estimates			
% Effect	Conc-%	95% LCL	95% UCL
50	0.000230769	0.000128205	0.000415385

Data Summary		Calculated Variate(A/B)							
Conc-%	Control Type	Count	Mean	Minimum	Maximum	SE	SD	A	B
0	Dilution Water	4	1.00000	1.00000	1.00000	0.00000	0.00000	20	20
0.0003		4	0.35000	0.00000	0.80000	0.06972	0.34157	7	20
0.0005		4	0.00000	0.00000	0.00000	0.00000	0.00000	0	20
0.0007		4	0.00000	0.00000	0.00000	0.00000	0.00000	0	20
0.001		4	0.00000	0.00000	0.00000	0.00000	0.00000	0	20

Data Detail											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1.00000	1.00000	1.00000	1.00000						
0.0003		0.00000	0.20000	0.40000	0.80000						
0.0005		0.00000	0.00000	0.00000	0.00000						
0.0007		0.00000	0.00000	0.00000	0.00000						
0.001		0.00000	0.00000	0.00000	0.00000						



5/4/2020

**To– Ecotiv Clean (Purgo formally Xtreme)– Yuri Ratner****Microbiological Test –****Chemical disinfectants and antiseptics - Quantitative carrier test  
For the evaluation of bactericidal and fungicidal activity for instruments  
used in the medical and food areas****1. Standard:**

The test was conducted based on Israeli Standard 1944, BS EN 14561  
"Evaluation of bactericidal activity" and AAMI TIR 12 (2010).

**Laboratory Number: 20034032**

**Sample description:** Disinfection spray- Purgo formally  
Xtreme solution (60 ml + 3.8 liter water – 2oz/1 gallon)

**Date sample received: 22/3/2020****Date Tested: 29/03/2020****Test Purpose:**

This test was conducted in order to define the anti microbial effectiveness of the  
disinfectant preparation (PURgo formally Xtreme) for surface disinfection.

**Inoculation:**

- 1.1 Stainless steel surfaces (4/4cm) were sterilized by steam.
- 1.2 The surfaces were inoculated with the following microorganisms (four  
surfaces for each microorganism) –

<i>Staphylococcus aureus</i>	ATCC 6538
<i>Pseudomonas aeruginosa</i>	ATCC 9027
<i>Escherichia coli</i>	ATCC 8739
<i>Aspergillus niger</i>	ATCC 16404
<i>Enterococcus faecalis</i>	ATCC 51299
<i>Lactobacillus plantrorum</i>	ATCC 14917
<i>Salmonella typhimurium</i>	ATCC 14028
<i>Enterobacter aerogenes</i>	ATCC 13048
<i>Candida albicans</i>	ATCC 10231

Page 1 of 4

**Remarks:**

1. The laboratory operates under organized working procedures which correlate to the international standard ISO/IEC 17025 in those disciplines where accreditation has been granted.
2. The microbiological tests are within the recognition framework of the Ministry of Health as published in the registrations.
3. The results are related only to the tested sample.
4. This document may be referred to in its entirety, and no part may be quoted or copied to other documents.
5. Sampling was provided by and is the sole responsibility of the customer.
6. The Israel Laboratory Accreditation Authority is not responsible for the test results.
7. The valid results are those of the most updated report.

טופס מס' 156, מהדורה 2 9.06



<i>Saccharomyces cerevisiae</i> ATCC 51299
--

<i>Listeria monocytogenes</i> ATCC 19115
--

The bacterial suspensions were diluted using soil (ATS - containing Proteins Healthmark (MI, US)) in order to give a final concentration of Bacteria of  $10^5$ - $10^6$  per/surface (about 100  $\mu$ l from each suspension was added to the soil according to cell turbidity).

- 1.3 One surface was not inoculated – negative control.
- 1.4 The surfaces were left to dry in biohazard hood for 30 minutes.

## 2. Test Procedure:

- 2.1 Two surfaces before disinfection and cleaning for each microorganism were placed aseptically into sterile cups. 100 ml were added to each sample (Neutralizing solution lot 16378) and vortexed for 1 minute and then the diluted sample was plated according to the pour plate technique using warm TSA (lot 816) or SDA (lot 816) or APT (819).
- 2.2 The plates were incubated for 72 hours at 30-35°C or 120 hours at 20-25 °C for yeasts and moulds. After incubation of the test plates, the Microorganisms were counted on each plate.
- 2.3 The remaining surfaces (two for each microorganism) were subjected to disinfection according to manufacturer's instructions (contact for 15 seconds according to manufactures instructions). One surface after disinfection was put into cups and 0.1 ml of Neutralizing solution was spread on each surface. The surfaces were then diluted with 10 ml (BPS+1% Tween 80 lot 16378) and vortexed for 1 minute.
- 2.4 Then the eluent was plated in the pour plate technique using TSA or SDA or APT.
- 2.5 The plates were incubated as defined in 2.1 and then the microbial count was determined per surface.

Page 2 of 4

### Remarks:

1. The laboratory operates under organized working procedures which correlate to the international standard ISO/IEC 17025 in those disciplines where accreditation has been granted.
2. The microbiological tests are within the recognition framework of the Ministry of Health as published in the registrations.
3. The results are related only to the tested sample.
4. This document may be referred to in its entirety, and no part may be quoted or copied to other documents.
5. Sampling was provided by and is the sole responsibility of the customer.
6. The Israel Laboratory Accreditation Authority is not responsible for the test results.
7. The valid results are those of the most updated report.

טופס מס' 156, מהדורה 2 9.06

### 3. Results:

Bacteria/Yeast/Mould	Before disinfection CFU/surface	After disinfection CFU/surface
<i>P. aeruginosa</i> ATCC 9027	8,600,000 9,000,000	60 50
<i>S.aureus</i> ATCC 6358	3,300,000 4,000,000	<10 <1
<i>E.coli</i> ATCC 8739	2,800,000 3,000,000	8 <10
<i>Aspergillus niger</i> ATCC 16404	3,400,000 4,000,000	380 42
<i>Enterococcus faecalis</i> ATCC 51299	3,600,000 4,000,000	260 31
<i>Lactobacillus plantrorum</i> ATCC 14917	2,800,000 3,000,000	<10 <1
<i>Salmonella typhimurium</i> ATCC 14028	16,400,000 15,000,000	360 56
<i>Enterobacter aerogenes</i> ATCC 13048	14,600,000 15,000,000	260 32
<i>Saccharomyces cerevisiae</i> ATCC 51299	2,900,000 3,000,000	160 260
<i>Listeria monocytogenes</i> ATCC 19115	8,300,000 10,000,000	<10 <1
<i>Candida albicans</i> ATCC 10231	18,600,000 20,000,000	30 80
NC	<10, <10	

Page 3 of 4

#### Remarks:

1. The laboratory operates under organized working procedures which correlate to the international standard ISO/IEC 17025 in those disciplines where accreditation has been granted.
2. The microbiological tests are within the recognition framework of the Ministry of Health as published in the registrations.
3. The results are related only to the tested sample.
4. This document may be referred to in its entirety, and no part may be quoted or copied to other documents.
5. Sampling was provided by and is the sole responsibility of the customer.
6. The Israel Laboratory Accreditation Authority is not responsible for the test results.
7. The valid results are those of the most updated report.

טופס מס' 156, מהדורה 2 9.06

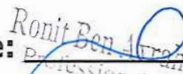


#### 4. Conclusion:

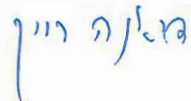
According to the test results, the disinfection using Ecotiv Clean disinfectant spray for 10 minutes in the presence of organic soil was able to reduce 4 magnitudes for *A.niger*, *E.fecalis* and *S.cerevisiae*, 5 -6 magnitudes for *P.aeruginosa*, *Candida albicans*, *E.coli*, *L.monocytogenes*, *L.plantarum*, *S.typhimurium*, *E.aerogenes* and *S.aureus* (at least 99.99% from the inoculated microorganism).

\*\*\*\*\*End of Test Results\*\*\*\*\*

Authorized Signature:

  
Ronit Ben Abraham PhD  
Professional Manager  
Microbial Laboratory

Preformed by: \_\_\_\_\_



Page 4 of 4

Remarks:

1. The laboratory operates under organized working procedures which correlate to the international standard ISO/IEC 17025 in those disciplines where accreditation has been granted.
2. The microbiological tests are within the recognition framework of the Ministry of Health as published in the registrations.
3. The results are related only to the tested sample.
4. This document may be referred to in its entirety, and no part may be quoted or copied to other documents.
5. Sampling was provided by and is the sole responsibility of the customer.
6. The Israel Laboratory Accreditation Authority is not responsible for the test results.
7. The valid results are those of the most updated report.

טופס מס' 156, מהדורה 2 9.06