

ProtectGuard

48-Hour Acute Toxicity Test Report

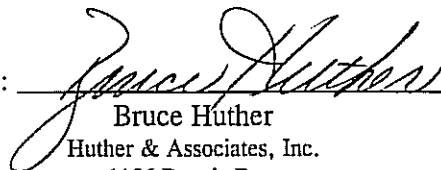
Ceriodaphnia dubia
Pimephales promelas

March 2008

Prepared for:

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48-HOUR LC50 PRODUCT REPORT

Client Guard Industry Project No: PT4577
Sample ProtectGuard Test Date April 2004

INTRODUCTION

A product identified as ProtectGuard was delivered to Huther and Associates Inc. on February 25, 2008. Two acute toxicity tests were requested: a static acute 48-hour definitive toxicity test using *Ceriodaphnia dubia* (water flea) and a static acute 48-hour definitive toxicity test using *Pimephales promelas* (fathead minnow). Test procedures followed recommended methods contained in "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition", EPA-821-R-02-012, October 2004 (Attachment A).

C. dubia and *P. promelas* are two freshwater aquatic indicator organisms frequently used to evaluate the potential toxicity of a compound or an effluent. The acute toxicity of a compound or effluent is generally measured using a multi-concentration, or definitive test, consisting of a control water and a minimum of five increasing concentrations of product added to control water. The tests are designed to provide dose-response information, expressed as the concentration that is lethal to 50% of the test organisms (LC50).

SAMPLE PREPARATION

The product was initially prepared for testing by adding a wide range of product concentrations to laboratory water (rangefinding tests). There was 100% survival to both species in the initial rangefinding tests. A second set of rangefinding tests were conducted using higher concentrations of product. Based on the results of the second set of rangefinding tests, definitive test solutions were conducted using a narrower range of concentrations.

The initial rangefinding test concentrations for both species were 50, 100, 200, 400, 800, 1600, 3200 and 6400 ml/L (ppm). The second set of rangefinding test concentrations were 5,000, 10,000, 20,000, 40,000, 60,000, 80,000 and 100,000 ml/L (ppm). The definitive test concentrations for both species were 4,000, 6,000, 8,000, 10,000, 12,000, 14,000, 16,000 and 18,000 ml/L (ppm).

Laboratory water/control water was distilled, deionized water reconstituted with reagent grade chemicals to a hardness of 160 mg/L as CaCO₃, an alkalinity of 110 mg/L and a pH of 8.20.

TEST DESIGN
Ceriodaphnia dubia

The 48-hour static, non-renewal, definitive *Ceriodaphnia dubia* test was conducted in 20 mL beakers containing 10 mL of test solution. The test was initiated March 5, 2008 following completion of the rangefinding tests. Five *C. dubia* neonates, less than 24-hours old, were added to each of the four replicate beakers per concentration. Neonates were fed a concentration of *Selenastrum capricornutum* plus cerophyll extract while in holding prior to test initiation. The test was conducted for 48-hours during which survival was recorded daily.

A control of four replicate beakers containing five *C. dubia* neonates each in laboratory water was conducted concurrently with the test. The test was completed on March 7, 2008. Survival data were statistically analyzed using the Trimmed Spearman-Kärber point estimate test to determine the lethal concentration to fifty percent (50%) of the test population (LC50).

RESULTS
Ceriodaphnia dubia

The following estimated LC50 value was determined for ProtectGuard:

| 48-Hour Definitive Test | | | | |
|-------------------------------|-----------|---------|------------------|------------|
| Conc. (ml/L) | # exposed | # alive | #dead | % survival |
| Control | 20 | 20 | 0 | 100.0 |
| 4,000 | 20 | 20 | 0 | 100.0 |
| 6,000 | 20 | 20 | 0 | 100.0 |
| 8,000 | 20 | 16 | 4 | 80.0 |
| 10,000 | 20 | 18 | 2 | 90.0 |
| 12,000 | 20 | 9 | 11 | 45.0 |
| 14,000 | 20 | 3 | 17 | 15.0 |
| 16,000 | 20 | 0 | 20 | 0.0 |
| 18,000 | 20 | 0 | 20 | 0.0 |
| Percent Spearman-Kärber | | | 0.0% | |
| Estimated LC50 (ml/L): | | | 11,271.83 | |
| 95% Upper C.L. (mg/L): | | | 12,066.41 | |
| 95% Lower C.L. (mg/L): | | | 10,529.57 | |

TEST DESIGN
Pimephales promelas

The 48-hour static, non-renewal, definitive *Pimephales promelas* test was conducted in 250 mL beakers containing 200 mL of test solution. The test was initiated March 5, 2008 following completion of the rangefinding tests. Ten *P. promelas* larvae were added to each of two replicate beakers per concentration. Larvae originated from laboratory cultures and were six days old at test initiation. Larvae were fed laboratory cultured *Artemia* nauplii during test exposure. The test was conducted for 48-hours during which survival was recorded daily.

A control of two replicate beakers containing ten *P. promelas* larvae each in laboratory water was conducted concurrently with the test. The test was terminated on March 7, 2008. Survival data were statistically analyzed using the Trimmed Spearman-Kärber point estimate test to determine the lethal concentration to fifty percent (50%) of the test population (LC50).

RESULTS

Pimephales promelas

The following estimated LC50 value was determined for ProtectGuard:

| 48-Hour Definitive Test | | | | |
|-------------------------------|-----------|---------|------------------|------------|
| Conc. (ml/L) | # exposed | # alive | #dead | % survival |
| Control | 20 | 20 | 0 | 100.0 |
| 4,000 | 20 | 20 | 0 | 100.0 |
| 6,000 | 20 | 20 | 0 | 100.0 |
| 8,000 | 20 | 20 | 0 | 100.0 |
| 10,000 | 20 | 20 | 0 | 100.0 |
| 12,000 | 20 | 10 | 10 | 50.0 |
| 14,000 | 20 | 1 | 19 | 5.0 |
| 16,000 | 20 | 0 | 20 | 0.0 |
| 18,000 | 20 | 0 | 20 | 0.0 |
| Percent Spearman-Kärber Trim: | | | 0.00% | |
| Estimated LC50 (ml/L): | | | 12,001.79 | |
| 95 % Upper C.L. (ml/L): | | | 12,493.42 | |
| 95 % Lower C.L. (ml/L): | | | 11,529.51 | |

DISCUSSION AND CONCLUSIONS

Ceriodaphnia dubia were determined to be the more sensitive species to ProtectGuard with an estimated 48-hour LC50 concentration of 11,271.93 ml/L. The estimated 48-hour LC50 concentration to *Pimephales promelas* was 12,001.79 ml/L.

Currently, there is no universal aquatic toxicity classification for chemicals. The US uses an aquatic toxicity classification system for pesticides while a proposed aquatic toxicity chemical classification system between the US and Europe is known as the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

The following Table compares both classification systems (Attachment B):

| GHS | US Pesticide Aquatic Toxicology Classification | |
|----------------|--|------------------|
| Classification | | LC50 (ml/L; ppm) |
| Category 1 | super | <0.01 |
| Category 1 | extreme | 0.01-0.10 |
| Category 1 | high | 0.11 - 1.0 |
| Category 2 | moderate | 1.1 - 10.0 |
| Category 3 | slight | 11.0 - 100.0 |
| | minimal | > 100.0 |

Neither of the classification systems address chemicals with LC50 values exceeding 1,000 ml/L. It is therefore concluded that even if the product was applied shortly before a rainfall run-off event or drift occurred during application, this product would pose minimal, if any, acute toxicity in the receiving body of water.

RAW DATA

48-HOUR CERIODAPHNIA DUBIA SURVIVAL

CLIENT: PROTECTGUARD RANGEFINDING

PROJECT#: PT4577

| CONC. (%) | NUMBER ORGANISMS, 0 HRS | | | | NUMBER ORGANISMS, 24 HRS | | | | NUMBER ORGANISMS, 48 HRS | | | | MEAN | CV% |
|------------|----------------------------|---|---|---|-----------------------------|---|---|---|-----------------------------|---|---|---|------|-----|
| | A | B | C | D | A | B | C | D | A | B | C | D | | |
| Con | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 50 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 100 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 200 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 400 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 800 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 1600 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 3200 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 6400 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| TECHNICIAN | JO | | | | LH | | | | H | | | | | |
| DATE/TIME | 2-16-07 1635 | | | | 2-27-08 1635 | | | | 2-28-08 1635 | | | | | |

48-HOUR PIMEPHALES PROMELAS SURVIVAL

CLIENT: Protect Guard

PROJECT#: PT4572 *Range finding*

| CONC. (%) | NUMBER ORGANISMS, 0 HRS | | NUMBER ORGANISMS, 24 HRS | | NUMBER ORGANISMS, 48 HRS | | MEAN | CV% |
|-----------------------|----------------------------|----|-----------------------------|----|-----------------------------|----|------|-----|
| | A | B | A | B | A | B | | |
| <i>ppm</i> Control | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 50 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 100 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 200 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 400 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 800 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 1600 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 3200 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 6400 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| TECHNICIAN | mm | | mm | | 20 | | | |
| DATE/TIME | 2-27-08 1525 | | 2-28-08 1525 | | 2-29-08 1525 | | | |

48-HOUR PIMEPHALES PROMELAS SURVIVAL

CLIENT: PROTECT GUARD HANEFIND 100 FT2

PROJECT#: PT 4522

| CONC. (%) | NUMBER ORGANISMS, 0 HRS | | NUMBER ORGANISMS, 24 HRS | | NUMBER ORGANISMS, 48 HRS | | MEAN | CV% |
|------------|----------------------------|----|-----------------------------|-----------------|-----------------------------|----|------|-----|
| | A | B | A | B | A | B | | |
| Con | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 5,000 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 10,000 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 20,000 | 10 | 10 | 0 ₁₀ | 0 ₁₀ | - | - | | |
| 40,000 | 10 | 10 | 0 ₁₀ | 0 ₁₀ | - | - | | |
| 60,000 | 10 | 10 | 0 ₁₀ | 0 ₁₀ | - | - | | |
| 80,000 | 10 | 10 | 0 ₁₀ | 0 ₁₀ | - | - | | |
| 100,000 | 10 | 10 | 0 ₁₀ | 0 ₁₀ | - | - | | |
| TECHNICIAN | mm | | mm | | mm | | | |
| DATE/TIME | 3-3-08 1325 | | 3-4-08 1325 | | 3-5-08 1325 | | | |

48-HOUR CERIODAPHNE DUBIA SURVIVAL

CLIENT: PROTECT GUARD RANGEFINDING #2

PROJECT#: PT4577

| CONC. (%) | NUMBER ORGANISMS, 0 HRS | | | | NUMBER ORGANISMS, 24 HRS | | | | NUMBER ORGANISMS, 48 HRS | | | | MEAN | CV% |
|------------|----------------------------|---|---|---|-----------------------------|----------------|----------------|----------------|-----------------------------|----------------|---|----------------|------|-----|
| | A | B | C | D | A | B | C | D | A | B | C | D | | |
| Con | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 5,000 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 10,000 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 3 ₂ | | |
| 20,000 | 5 | 5 | 5 | 5 | 3 ₂ | 2 ₃ | 0 ₅ | 2 ₃ | 0 ₃ | 0 ₂ | - | 0 ₂ | | |
| 40,000 | 5 | 5 | 5 | 5 | 0 ₅ | 0 ₅ | 0 ₅ | 0 ₅ | - | - | - | - | | |
| 60,000 | 5 | 5 | 5 | 5 | 0 ₅ | 0 ₅ | 0 ₅ | 0 ₅ | - | - | - | - | | |
| 80,000 | 5 | 5 | 5 | 5 | 0 ₅ | 0 ₅ | 0 ₅ | 0 ₅ | - | - | - | - | | |
| 100,000 | 5 | 5 | 5 | 5 | 0 ₅ | 0 ₅ | 0 ₅ | 0 ₅ | - | - | - | - | | |
| TECHNICIAN | mm | | | | UH | | | | UH | | | | | |
| DATE/TIME | 3-3-08 1120 | | | | 3-4-08 1120 | | | | 3-5-08 1120 | | | | | |

48- Hour Acute Toxicity Summary Form

Permittee: PROTECT GUARD
 Outfall: RANGEFINDING #2
 Lab ID No.: PTV327
 Begin Time/ Date 3/3/08

Organism: Ceriodaphnia dubia
 End Date/Time 3/5/08 *DK*

| Date | Time | Temp | Samp. No. | pH (mg/L) of Solution | | | | | | | Analyst |
|--------|-------|------|-----------|-----------------------|-------|--------|--------|--------|--------|--------|---------|
| | | | | Con | 5,000 | 10,000 | 20,000 | 40,000 | 60,000 | 80,000 | |
| 3/3/08 | Start | | 1 | 812 | 814 | 811 | 808 | 800 | 793 | 786 | AC |
| 3-4 | 24-Hr | | 1 | | | | | | | | |
| 3-4 | Renew | | 1 | | | | | | | | |
| 3-5 | 48-Hr | | 1 | 802 | 794 | 787 | 789 | DEAD | DEAD | DEAD | AC |

| Date | Time | Temp | Samp. No. | DO (mg/L) of Solution | | | | | | | Analyst |
|--------|-------|------|-----------|-----------------------|-------|--------|--------|--------|--------|--------|---------|
| | | | | Con | 5,000 | 10,000 | 20,000 | 40,000 | 60,000 | 80,000 | |
| 3/3/08 | Start | | 1 | 821 | 823 | 814 | 801 | 798 | 792 | 786 | AC |
| 3-4 | 24-Hr | | 1 | | | | | | | | |
| 3-4 | Renew | | 1 | | | | | | | | |
| 3-5 | 48-Hr | | 1 | 715 | 705 | 707 | 711 | DEAD | DEAD | DEAD | AC |

Begin Time/ Date 3/3/08

Organism: Limnithales promelas
 End Date/Time _____

| Date | Time | Temp | Samp. No. | pH (mg/L) of Solution | | | | | | | Analyst |
|--------|-------|------|-----------|-----------------------|-------|--------|--------|--------|--------|--------|---------|
| | | | | Con | 5,000 | 10,000 | 20,000 | 40,000 | 60,000 | 80,000 | |
| 3/3/08 | Start | | 1 | 812 | 814 | 811 | 808 | 800 | 793 | 786 | AC |
| 3-4 | 24-Hr | | 1 | | | | | | | | |
| 3-4 | Renew | | 1 | 826 | | | | | | | |
| 3-5 | 48-Hr | | 1 | 826 | 812 | 795 | DEAD | DEAD | DEAD | DEAD | AC |

| Date | Time | Temp | Samp. No. | DO (mg/L) of Solution | | | | | | | Analyst |
|--------|-------|------|-----------|-----------------------|-------|--------|--------|--------|--------|--------|---------|
| | | | | Con | 5,000 | 10,000 | 20,000 | 40,000 | 60,000 | 80,000 | |
| 3/3/08 | Start | 1 | 1 | 821 | 823 | 814 | 801 | 798 | 792 | 786 | AC |
| 3-4 | 24-Hr | | 1 | | | | | | | | |
| 3-4 | Renew | | 1 | | | | | | | | |
| 3-5 | 48-Hr | | 1 | 735 | 690 | 625 | DEAD | DEAD | DEAD | DEAD | AC |

| Date | Samp. No. | pH | DO | Hardness (mg/L CaCO ₃) | Alkalinity (mg/L CaCO ₃) | Conduct (umhos/cm) | Resid Cl ₂ (mg/L) | Dechlor (mL Na ₂ S ₂ O ₃ mg/L) | Analyst |
|------|-----------|------|------|------------------------------------|--------------------------------------|--------------------|------------------------------|---|---------|
| 3/3 | 1 | / | / | / | / | / | / | / | / |
| | 2 | / | / | / | / | / | / | / | / |
| 3/3 | Con. | 8.12 | 8.21 | 160 | 110 | 760 | | | |

48-HOUR CERIODAPHNIA DUBIA SURVIVAL

CLIENT: Protect Guard

PROJECT#: P74577 Definitive Test

| CONC. (%) | NUMBER ORGANISMS, 0 HRS | | | | NUMBER ORGANISMS, 24 HRS | | | | NUMBER ORGANISMS, 48 HRS | | | | MEAN | CV% |
|------------|----------------------------|---|---|---|-----------------------------|---|----------------|----------------|-----------------------------|----------------|----------------|----------------|------|-----|
| | A | B | C | D | A | B | C | D | A | B | C | D | | |
| CON | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 4,000 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 6,000 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| 8,000 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 ₁ | 4 ₁ | 3 ₂ | 5 | | |
| 10,000 | 5 | 5 | 5 | 5 | 4 ₁ | 5 | 4 ₁ | 5 | 4 | 5 | 4 | 5 | | |
| 12,000 | 5 | 5 | 5 | 5 | 5 | 5 | 3 ₂ | 3 ₂ | 4 | 3 ₂ | 3 | 2 ₁ | | |
| 14,000 | 5 | 5 | 5 | 5 | 2 | 3 | 2 | 3 | 1 ₁ | 0 ₃ | 1 ₁ | 1 ₂ | | |
| 16,000 | 5 | 5 | 5 | 5 | 0 | 0 | 0 | 2 | - | - | - | 0 ₂ | | |
| 18,000 | 5 | 5 | 5 | 5 | 2 | 1 | 4 | 2 | 0 ₂ | 0 ₁ | 0 ₄ | 0 ₂ | | |
| 20,000 | 5 | 5 | 5 | 5 | 5 | 3 | 4 | 1 | 0 ₅ | 0 ₃ | 0 ₄ | 0 ₁ | | |
| TECHNICIAN | MM | | | | ZO | | | | MM | | | | | |
| DATE/TIME | 3-5-08 1615 | | | | 3-6-08 1615 | | | | 3-7-08 1615 | | | | | |

48-HOUR PIMEPHALES PROMELAS SURVIVAL

CLIENT: Protect Guard

PROJECT#: PT4577 Definitive Test

| CONC. (%) | NUMBER ORGANISMS, 0 HRS | | NUMBER ORGANISMS, 24 HRS | | NUMBER ORGANISMS, 48 HRS | | MEAN | CV% |
|------------|----------------------------|----|-----------------------------|-----|-----------------------------|----|------|-----|
| | A | B | A | B | A | B | | |
| Con | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 4,000 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 6,000 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 8,000 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 10,000 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| 12,000 | 10 | 10 | 55 | 64 | 5 | 51 | | |
| 14,000 | 10 | 10 | 28 | 010 | 1 | - | | |
| 16,000 | 10 | 10 | 010 | 010 | - | - | | |
| 18,000 | 10 | 10 | 010 | 010 | - | - | | |
| TECHNICIAN | ZJ | | ZJ | | ZJ | | | |
| DATE/TIME | 3-5-08 1545 | | 3-6-08 1545 | | 3-7-08 1545 | | | |

48-Hour Acute Toxicity Summary Form

Permittee: Protect Guard
 Outfall: DEFINITIVE TEST
 Lab ID No.: PT4377
 Begin Time/Date 3/5/08 1545

Organism: Fat
 End Date/Time 3/7/08 1545

| Date | Time | Temp | Samp. No. | pH (mg/L) of Solution | | | | | | | Analyst |
|------|-------|------|-----------|-----------------------|-------|-------|--------|--------|--------|--------|---------|
| | | | | CON | 6,000 | 8,000 | 10,000 | 12,000 | 14,000 | 16,000 | |
| 3-5 | Start | 25.0 | 1 | 820 | 816 | 815 | 812 | 814 | 814 | 813 | AC |
| 3-6 | 24 Hr | 24.0 | 1 | | | | | | | | |
| 3-6 | Renew | 25.0 | 2 | | | | | | | | |
| 3-7 | 48 Hr | 24.0 | 2 | 820 | 832 | 824 | 821 | 823 | 823 | 822 | MM |

| Date | Time | Temp | Samp. No. | DO (mg/L) of Solution | | | | | | | Analyst |
|------|-------|------|-----------|-----------------------|-------|-------|--------|--------|--------|--------|---------|
| | | | | CON | 6,000 | 8,000 | 10,000 | 12,000 | 14,000 | 16,000 | |
| 3-5 | Start | 25.0 | 1 | 756 | 756 | 750 | 727 | 734 | 732 | 728 | AC |
| 3-6 | 24 Hr | 24.0 | 1 | | | | | | | | |
| 3-6 | Renew | 25.0 | 2 | | | | | | | | |
| 3-7 | 48 Hr | 24.0 | 2 | 756 | 695 | 687 | 681 | 686 | 689 | 691 | MM |

Organism: Cerio
 Begin Time/Date 3/5/08 1615
 End Date/Time 3/7/08 1615

| Date | Time | Temp | Samp. No. | pH (mg/L) of Solution | | | | | | | Analyst |
|------|-------|------|-----------|-----------------------|-------|-------|--------|--------|--------|--------|---------|
| | | | | CON | 6,000 | 8,000 | 10,000 | 12,000 | 14,000 | 16,000 | |
| 3-5 | Start | 25.0 | 1 | 820 | 816 | 815 | 812 | 814 | 814 | 813 | AC |
| 3-6 | 24 Hr | 24.0 | 1 | | | | | | | | |
| 3-6 | Renew | 25.0 | 2 | | | | | | | | |
| 3-7 | 48 Hr | 24.0 | 2 | 820 | 832 | 824 | 821 | 823 | 823 | 822 | MM |

| Date | Time | Temp | Samp. No. | DO (mg/L) of Solution | | | | | | | Analyst |
|------|-------|------|-----------|-----------------------|-------|-------|--------|--------|--------|--------|---------|
| | | | | CON | 6,000 | 8,000 | 10,000 | 12,000 | 14,000 | 16,000 | |
| 3-5 | Start | 25.0 | 1 | 756 | 756 | 750 | 727 | 734 | 732 | 728 | AC |
| 3-6 | 24 Hr | 24.0 | 1 | | | | | | | | |
| 3-6 | Renew | 25.0 | 2 | | | | | | | | |
| 3-7 | 48 Hr | 24.0 | 2 | 756 | 694 | 687 | 681 | 686 | 689 | 691 | MM |

| Date | Samp. No. | pH | DO | Hardness (mg/L CaCO ₃) | Alkalinity (mg/L CaCO ₃) | Conduct (umhos/cm) | Resid Cl ₂ (mg/L) | Dechlor (mg Na ₂ S ₂ O ₃ /mg Cl ₂) | Analyst |
|------|-----------|------|------|------------------------------------|--------------------------------------|--------------------|------------------------------|---|---------|
| | 1 | / | / | / | / | / | / | / | / |
| | 2 | / | / | / | / | / | / | / | / |
| | Con. | 8.20 | 7.56 | 160 | 110 | 768 | | | |

48- Hour Acute Toxicity Summary Form

Organism: Fat

Permittee: Protect Guard

Outfall: DEFINITIVE TEST

Lab ID No.: PT 4577

Begin Time/ Date 3/5/08 1545

End Date/Time 3/7/08 1545

| Date | Time | Temp | Samp No. | pH (mg/L) of Solution | | | Analyst |
|------|-------|------|----------|-----------------------|--------|-------|---------|
| | | | | 18,000 | 20,000 | 4,000 | |
| 3-5 | Start | 25.0 | 1 | 812 | 809 | 816 | AC |
| 3-6 | 24-Hr | 24.0 | 1 | | | | |
| 3-6 | Renew | 25.0 | 2 | | | | MM |
| 3-7 | 48-Hr | 24.0 | 2 | 820 | 817 | 819 | MM |

| Date | Time | Temp | Samp No. | DO (mg/L) of Solution | | | Analyst |
|------|-------|------|----------|-----------------------|--------|-------|---------|
| | | | | 18,000 | 20,000 | 4,000 | |
| 3-5 | Start | 25.0 | 1 | 729 | 720 | 756 | AC |
| 3-6 | 24-Hr | 24.0 | 1 | | | | |
| 3-6 | Renew | 25.0 | 2 | | | | MM |
| 3-7 | 48-Hr | 24.0 | 2 | 699 | 689 | 697 | MM |

Organism: Cerio

End Date/Time 3/7/08 1615

Begin Time/ Date 3/5/08 1615

| Date | Time | Temp | Samp No. | pH (mg/L) of Solution | | | Analyst |
|------|-------|------|----------|-----------------------|--------|-------|---------|
| | | | | 18,000 | 20,000 | 4,000 | |
| 3-5 | Start | 25.0 | 1 | 812 | 809 | 816 | AC |
| 3-6 | 24-Hr | 24.0 | 1 | | | | |
| 3-6 | Renew | 25.0 | 2 | | | | MM |
| 3-7 | 48-Hr | 24.0 | 2 | 820 | 817 | 819 | MM |

| Date | Time | Temp | Samp No. | DO (mg/L) of Solution | | | Analyst |
|------|-------|------|----------|-----------------------|--------|-------|---------|
| | | | | 18,000 | 20,000 | 4,000 | |
| 3-5 | Start | 25.0 | 1 | 729 | 720 | 756 | AC |
| 3-6 | 24-Hr | 24.0 | 1 | | | | |
| 3-6 | Renew | 25.0 | 2 | | | | MM |
| 3-7 | 48-Hr | 24.0 | 2 | 699 | 689 | 697 | MM |

| Date | Samp No. | pH | DO | Hardness mg/L CaCO3 | Alkalinity mg/L CaCO3 | Conduct umhos/cm | Resist. Cl2 mg/L | Decolor (ml) Na2S2O3 mg/L | Analyst |
|------|----------|------|-----|------------------------|--------------------------|---------------------|---------------------|---------------------------------|---------|
| | 1 | / | / | / | / | / | / | / | / |
| | 2 | / | / | / | / | / | / | / | / |
| | Con. | 8.20 | 756 | 160 | 110 | | | | |

LC50 DETERMINATIONS

TRIMMED SPEARMAN-KARBER METHOD. VERSION 1.5

DATE: MARCH 20 TEST NUMBER: 1 DURATION: 48 H
TOXICANT : PROTECTGUARD
SPECIES: CERIODAPHNIA DUBIA

| RAW DATA: | Concentration (PPM) | Number Exposed | Mortalities |
|-----------|------------------------|-------------------|-------------|
| --- | .00 | 20 | 0 |
| ---- | 6000.00 | 20 | 0 |
| | 8000.00 | 20 | 4 |
| | *****10,000 | 20 | 2 |
| | *****12,000 | 20 | 11 |
| | *****14,000 | 20 | 17 |
| | *****16,000 | 20 | 20 |

BN

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 11271.83
 95% LOWER CONFIDENCE: 10529.57
 95% UPPER CONFIDENCE: 12066.41

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.



TRIMMED SPEARMAN-KARBER METHOD. VERSION 1.5

DATE: MARCH 20 TEST NUMBER: 1 DURATION: 48 H
TOXICANT : PROTECTGUARD
SPECIES: PIMEPHALES PROMELAS

| RAW DATA: | Concentration (PPM) | Number Exposed | Mortalities |
|-----------|----------------------------|-------------------|-------------|
| --- | .00 | 20 | 0 |
| --- | 8000.00 | 20 | 0 |
| --- | ***** 10,000 | 20 | 0 |
| --- | ***** 12,000 | 20 | 10 |
| --- | ***** 14,000 | 20 | 19 |
| --- | ***** 16,000 ^{RA} | 20 | 20 |

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 12001.79
95% LOWER CONFIDENCE: 11529.51
95% UPPER CONFIDENCE: 12493.42
