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## **Texas Plano Fresh Water Minnow Test With Cities Physical Engineer For Storm Drain Protection**

The OSEI Corporation CEO Mr. Steven Pedigo and team, performed a demonstration of how OSE II works and protects the environment, especially any oil or fuel spill where OSE II is applied to these types of materials, would break down their molecular structure, Reducing the toxicity, Eliminating Flammability, reducing Adhesion properties, and cause the oil or fuel to float on waters surface, protecting the water column where 60% of marine species live.

A fresh water test was run on fathead minnows for the physical engineer in Plano, Texas, USA. We were attempting to prove that hydrocarbons which have had OSEII applied to them and then washed in the storm drain would not add any toxicity to the storm drain.

One half gallon of gasoline was poured onto a concrete surface, where ••• gallon of OSE II (pre-diluted 100 to 1 was immediately applied. The treated gasoline was allowed to set for two (2) minutes at which time two (2) gallons of fresh water were used to wash this effluent into a catch basin. Approximately 1 ••• gallons were recovered and sent to Bio-Aquatic Laboratory.

Bio-Aquatic Laboratory performed a Static 48 Definitive Toxicity Test using Fathead Minnows (*Pimphales promeas*). The LC50 was 9,300 mg/L which is a relatively low toxicity level. This test shows that OSE II when applied to a toxic constituent rapidly reduces toxicity. This detoxifying action of OSE II limits the toxicity of a spill to marine organisms, and will allow Mother Nature's Bacteria to rapidly attack this detoxified spill. The rapid detoxification of a spill shows that OSE II is a beneficial tool for first response cleanup for a spill. This test also shows that if OSE II is used to clean up a parking lot and washed into the storm drain there would be no adverse environmental impact.

This helped alleviate the physical engineer's concerns for adding anything toxic to the storm drain and ultimately to a creek, river or lake. This test shows that using OSEII would help reduce the toxicity to storm drains from rain water runoff. If OSEII is used periodically to clean the parking lots allowing the sites in Plano to stay within its NPDES permitted discharge levels.

This also shows fire departments as well, who utilize OSE II for the elimination of flammability for fuels and solvents spilled, to also clean up the road area, addressing the environment, and rendering the spilled material non toxic, even when washed into storm drains.

This test also shows that if OSE II is used to clean up a parking lot or emergency spill, and washed into the storm drain there would be no adverse environmental impact.

Steven Pedigo

CEO OSEI Corporation

See Minnow test starting on the next page.

**OSEI CORPORATION OSE II/GASOLINE/WATER Toxicity Test Report**

DECEMBER 7, 1991

**BIO-AQUATIC TESTING, INC.**

Prepared by: \_\_\_\_\_ David Smith,  
Aquatic Toxicologist125

**BIO-AQUATIC TESTING, INC.**

1555 Valwood Parkway, Ste. 100 Carrollton, Texas 75006  
Tel: (214) 247-5928  
Fax: (214) 241-4474 **TOXICITY TEST REPORT – ACUTE**

Client . . . . . OSEI Corporation Laboratory I.D. . . . . BO-12- 91-2239  
Sample . . . . . OSE II/Gasoline/Water Date . . . . . December 7, 1991

Results: The 48-hour LC50 for *Pimephales promelas* exposed to a mixture of OSE II, gasoline, and water was 9,300 mg/L.

**SAMPLE  
COLLECTION**

**CHEMICAL MEASUREMENTS TEST PROCEDURES**

*Pimephales promelas*

Approximately one and a half gallons of runoff grab sample from an OSEI Corporation product demonstration was delivered to Bio-Aquatic Testing on December 5, 1991. The sample was manually collected by OSEI personnel. One toxicity test was requested: a static 48-hour definitive toxicity test using the fathead minnow (*Pimephales promelas*).

The sample was analyzed for residual chlorine (EPA Method 330.1, Amperometric Titration Method) and was determined to contain <0.10 mg/L. Sample and laboratory dilution water pH, temperature, conductivity, hardness, alkalinity and D.O. were analyzed and recorded daily.

The 48-hour fathead minnow larval survival test was initiated at 1450 hours, December 6, 1991. Five concentrations were established for testing (200 mg/L, 800 mg/L, 3,000 mg/L, 9,000 mg/L, and 30,000 mg/L) utilizing reconstituted distilled, deionized water as dilution water. The test was set up using distilled water rinsed 500 mL plastic cups as test chambers. Four replicate cups containing five organisms each in 250 mL of test solution were used per dilution. All organisms used were laboratory reared and less than 24 hours old at test initiation. The test was allowed to proceed for 48 hours during which mortality was recorded daily.

A control of four replicate chambers containing five organisms each in 100% synthetic laboratory water was conducted concurrently with the test. There was 100% survival in the control. Data on surviving organisms as well as water quality measurements were recorded on the data sheet. The

test ended at 1450 hours, December 8, 1991. The acute toxicity data analysis program provided by the EPA was employed to determine the LC50 values.126

## LC50 RESULTS

### *Pimephales promelas*

#### SUMMARY

LC50 value calculated using the Binomial Method: CONC. (mg/L) # EXPOSED # DEAD %  
DEAD BINOMIAL %

30,000 9,000 3,000 800 200

20

6

1

0

0

100

30

5

0

0.0001

5.7659

0.0020

0.0001

0.0001

The Binomial Test shows that 3,000 and 30,000 can be used as statistically sound conservative 95 percent confidence limits since the actual confidence level associated

with these limits is 99.99791 percent.

An approximate LC50 for this set of data is 11,800 mg/L.

LC50 value calculated using the Trimmed Spearman-Kärber Method:

Trim Var. of Ln Est. LC50 95% Conf. Limits

0.00% 0.17396D-01 9,300 mg/L 7,100 to 12,100 mg/L

The 48-hour LC50 for *Pimephales promelas* exposed to a mixture of OSE II, gasoline, and water was 9,300 mg/L.

# BIO-AQUATIC TESTING, INC.

## 48 – HOUR *PIMEPHALES PROMELAS* ACUTE TOXICITY TEST

CLIENT OSEI Corporation BEGIN DATE 12/06/91  
SAMPLE OSE II, Gasoline, Water END DATE 12/08/91  
LAB ID # **BO-12-91-2239B** TEST ORGANISM *Pimephales promelas* DATE COLLECTED  
12/05/91 TEST TEMPERATURE (°C) 25.0 ± 1 DATE RECEIVED 12/05/91 PHOTO PERIOD  
16 hour light / 8 hour dark SAMPLE TYPE Grab LIGHT INTENSITY 75 FT-C  
TEST TYPE Acute ANALYST W. Smith

### EFFLUENT MEASUREMENTS

D.O. @ 30,000 mg/L, 8.6/6.6  
pH @ 30,000, 8.3/8.4  
CONDUCTIVITY @ 30,000 (µMHOS) 500  
HARDNESS (mg/L as CaCO<sub>3</sub>) 272.4 ALKALINITY (mg/L as CaCO<sub>3</sub>) 625.0

### DECHLORINATION

RESIDUAL Cl<sub>2</sub> (mg/L) <0.10 ANALYSIS METHOD Amperometric Titration Method (330.1)  
DECHLORINATION REAGENT Not Applicable

### DILUTION WATER MEASUREMENTS

D.O. @ 100% (mg/L), 8.6/6.9  
pH @ 100% 8.4/8.3  
RECEIVING WATER DILUTION WATER Laboratory adjusted  
HARDNESS (mg/L as CaCO<sub>3</sub>) 160.0 ALKALINITY (mg/L as CaCO<sub>3</sub>) 107.0 Recorded at the beginning  
and end of each 24-hour exposure period.

x % Surv.

### SURVIVAL SUMMARY

x LIVE PER CONC

100  
100  
100  
95  
70

0  
%

EFFLUENT CONC Control 200 mg/L 800 mg/L

3,000 mg/L

9,000 mg/L

30,000 mg/L NUMBER LIVE PER REP START 24 HOURS 48 HOURS

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