Enzymatic Bioremediation of Petroleum Hydrocarbons

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Characteristics of Enzymatic Biotreatment of Oil Spills

- Biodegrades crude oil rapidly into smaller components and finally into CO_2 and H_2O
- Non-toxicity - demonstrated on fw and marine organisms – fish, plankton, and bacteria
- Non-toxic to humans
- Stimulates population growth in naturally occurring bacteria
- Works to completion within a period of 4-6 weeks – mechanism of action protects shorelines, beaches, marine life and human health within hours.

Four Types of Remediation for Oil Spills

NCP Product Types

- Dispersants
- Nutrient Additives
- Microbiological Cultures
- Enzyme Activities
  - Example – OSE-II®

Mechanism of Enzyme Bioremediation Action

- Contains

  - Bio-surfactants
  - Enzymes
  - Nutrients

Testin
g

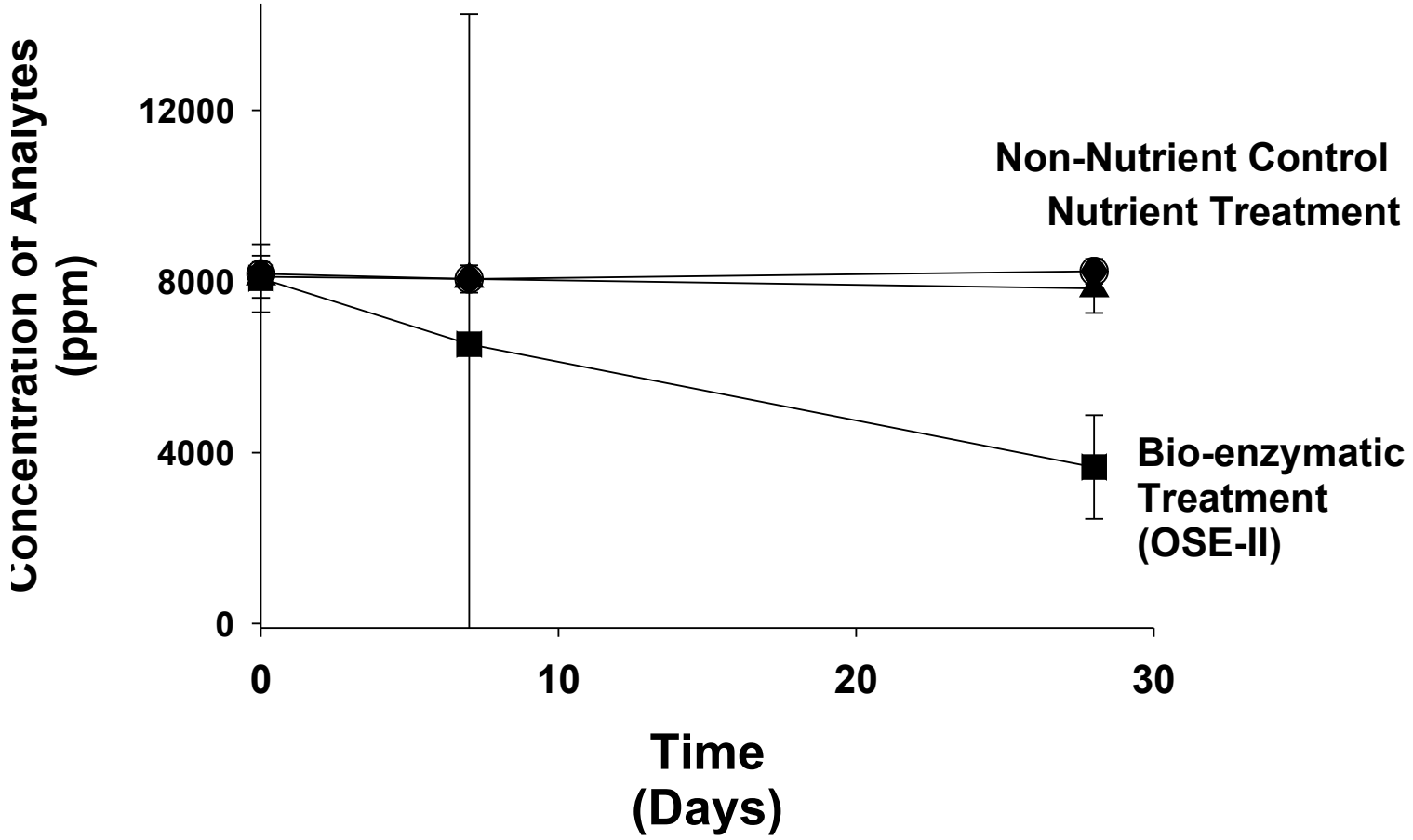
- Tests performed by numerous intl. govt. agencies and independent labs

- Non-Toxic

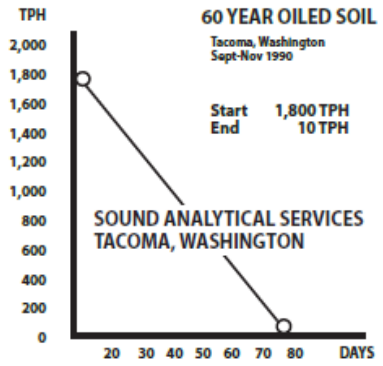
  - Safe for marine and aquatic organisms at effective applications levels
  - No specific precautions need be taken
  - No vapors emitted; thus, no inhalation problems
  - Safe for humans (OSHA)
  - No special handling or protective equipment or clothing required

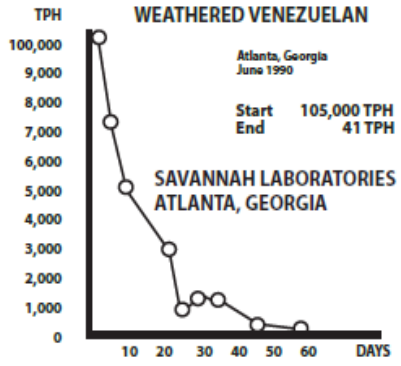
Efficacy

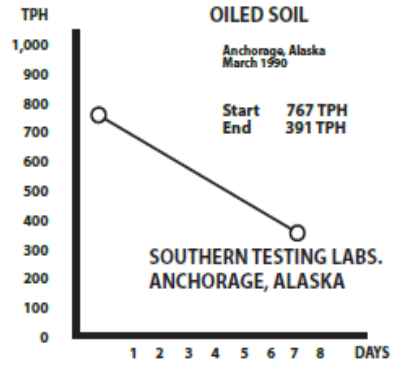
Degradation of 96 Analytes through Time Using a Bioenzymatic treatment (OSE-II) *vs.* Nutrient Controls

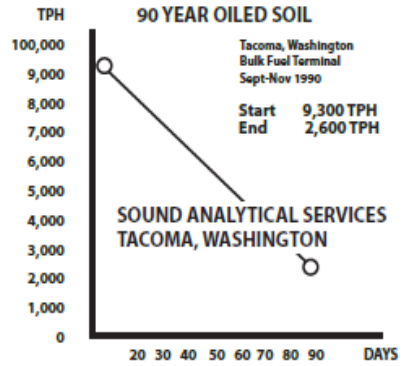


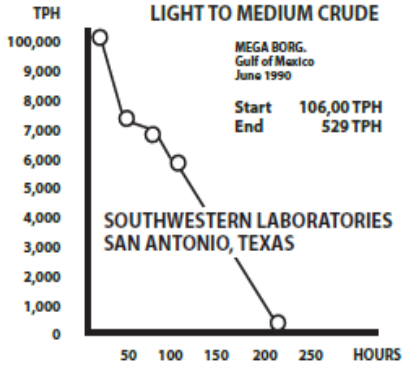
TPH BIODEGRADATION TESTS OF OSE II PERFORMED BY INDEPENDENT LABORATORIES

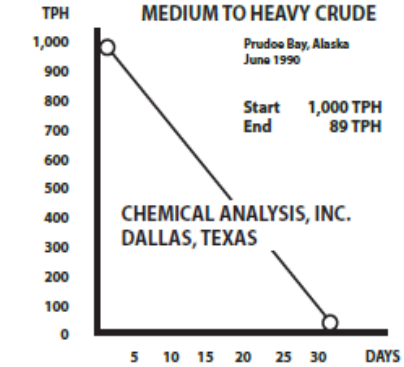










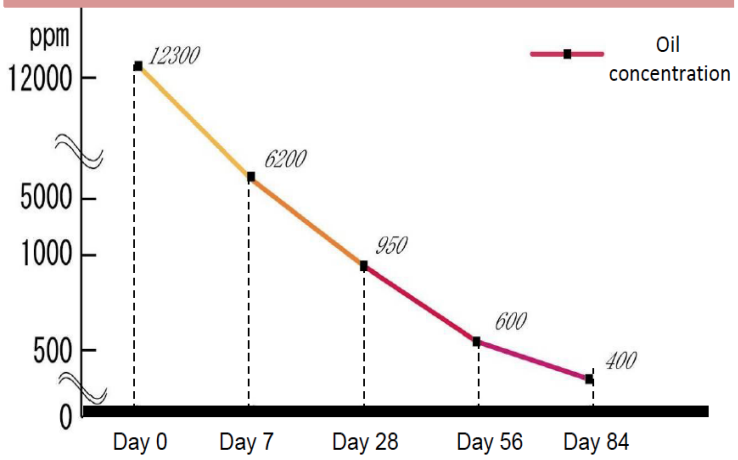


TPH Biodegradation Tests Using Enzymatic Treatment of Crude Oil Performed by Independent Labs (OSE-II)

Data : Oil decompose rate of OSEII bio stimulation

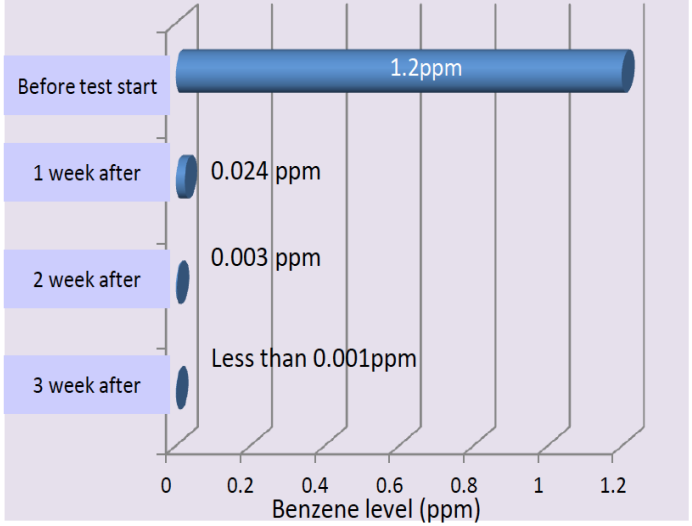
Analysis By University of Yamanashi (JAPAN)

Proceedings oil decompose

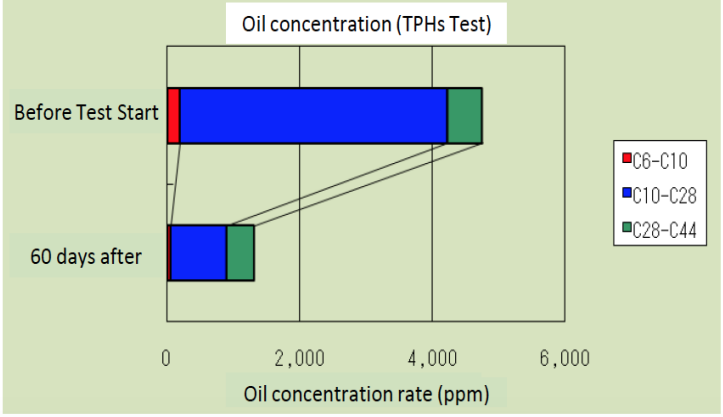


About ppm (parts per million) :
ppm is rate of contamination level of each soil.
We multiply amount of soil by ppm.
For Example: If the rate is 12300ppm from amount of 3000kg soil which means contain oil about 37kg (1.23%).
*Usually most of pollution rate is 1000-7000ppm.

Proceedings Benzene level



Proceedings each oil decompose

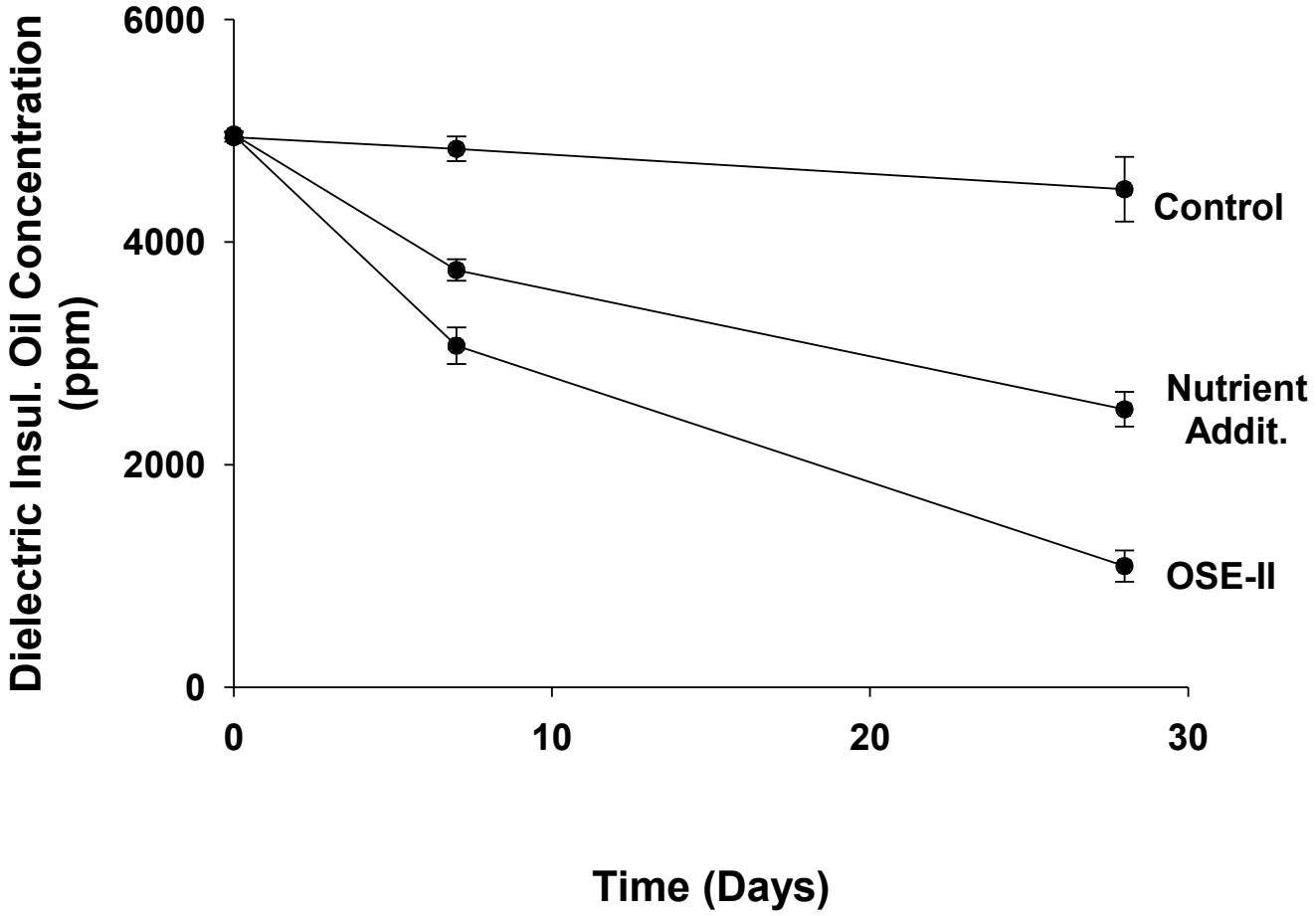


TPH = Total Petroleum Hydrocarbon

TPH include hexane, benzene, toluene, xylenes, naphthalene, and fluorene, other constituents of gasoline, of jet fuels, of mineral oils, and of other petroleum products.

(University of Yamanashi, Japan

Enzymatic Bioremediation
(OSE-II)
Dissipation of Spilled Dielectric Insulating Oil
(by GC/MS)
28 Days

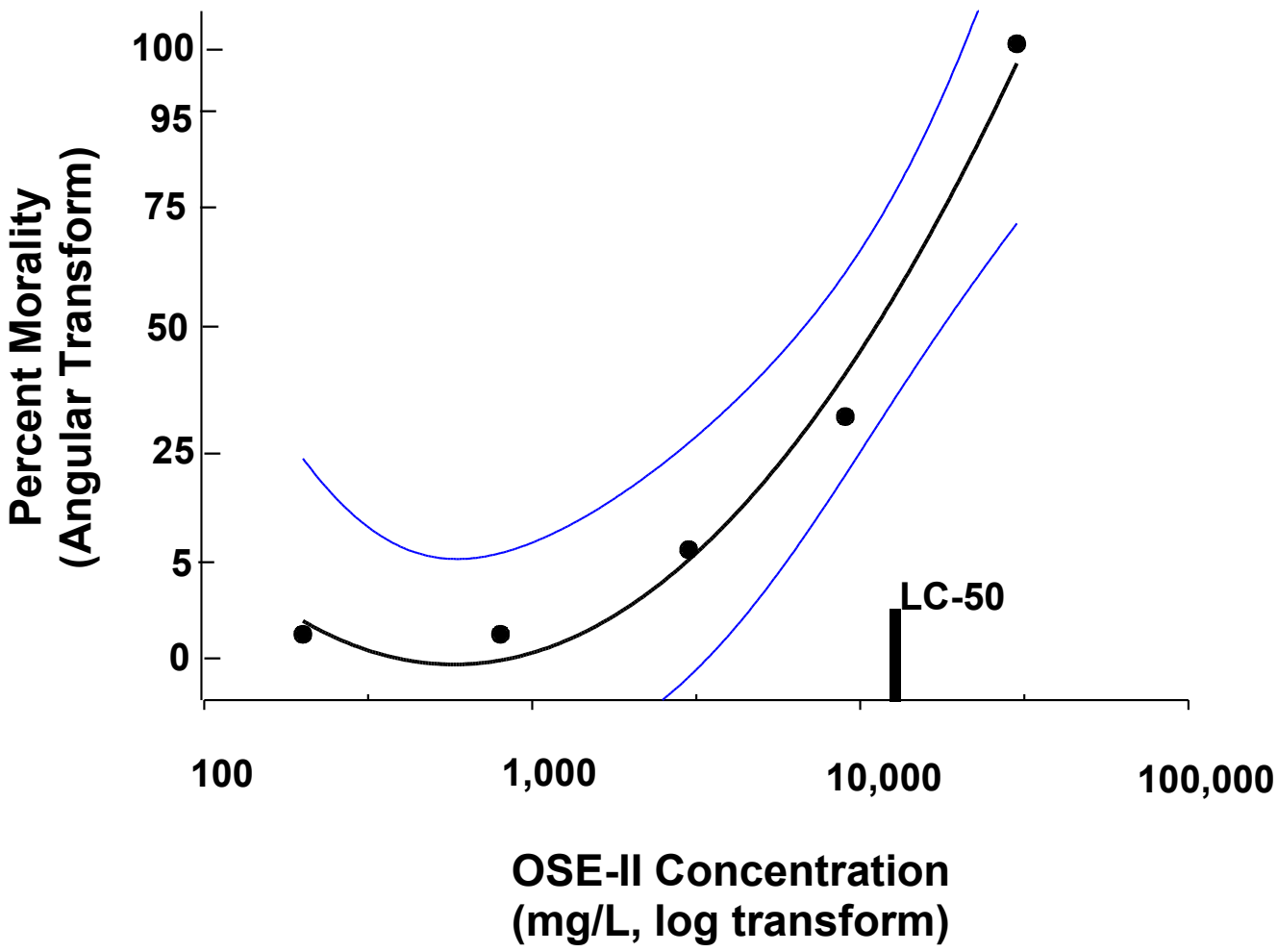


(U.S. Dept. Interior, BOEM; and
Dept. Envtl. Sci., LA State Univ.)

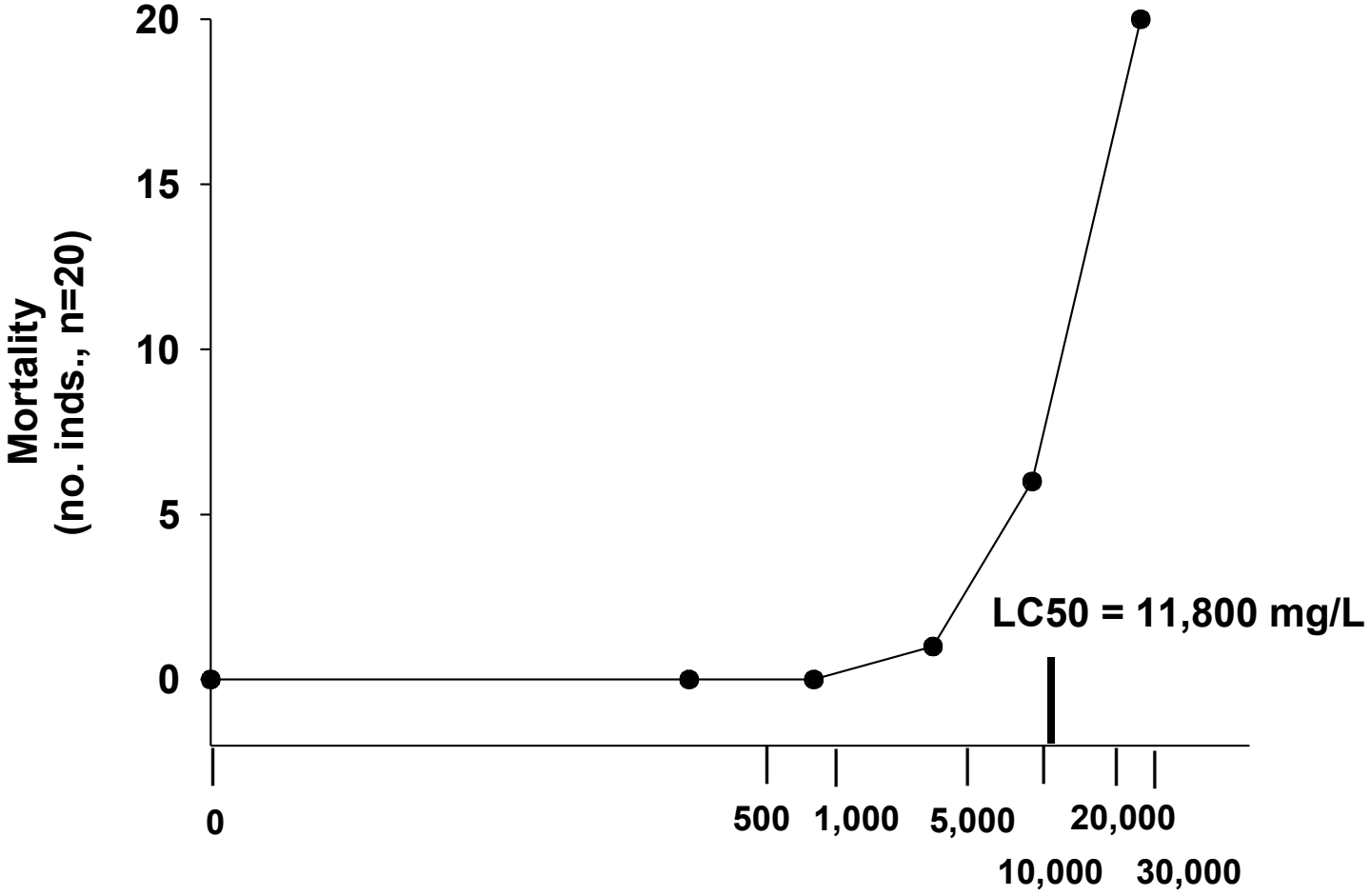
Toxicity

Pimephales promelas
(Fathead Minnow)

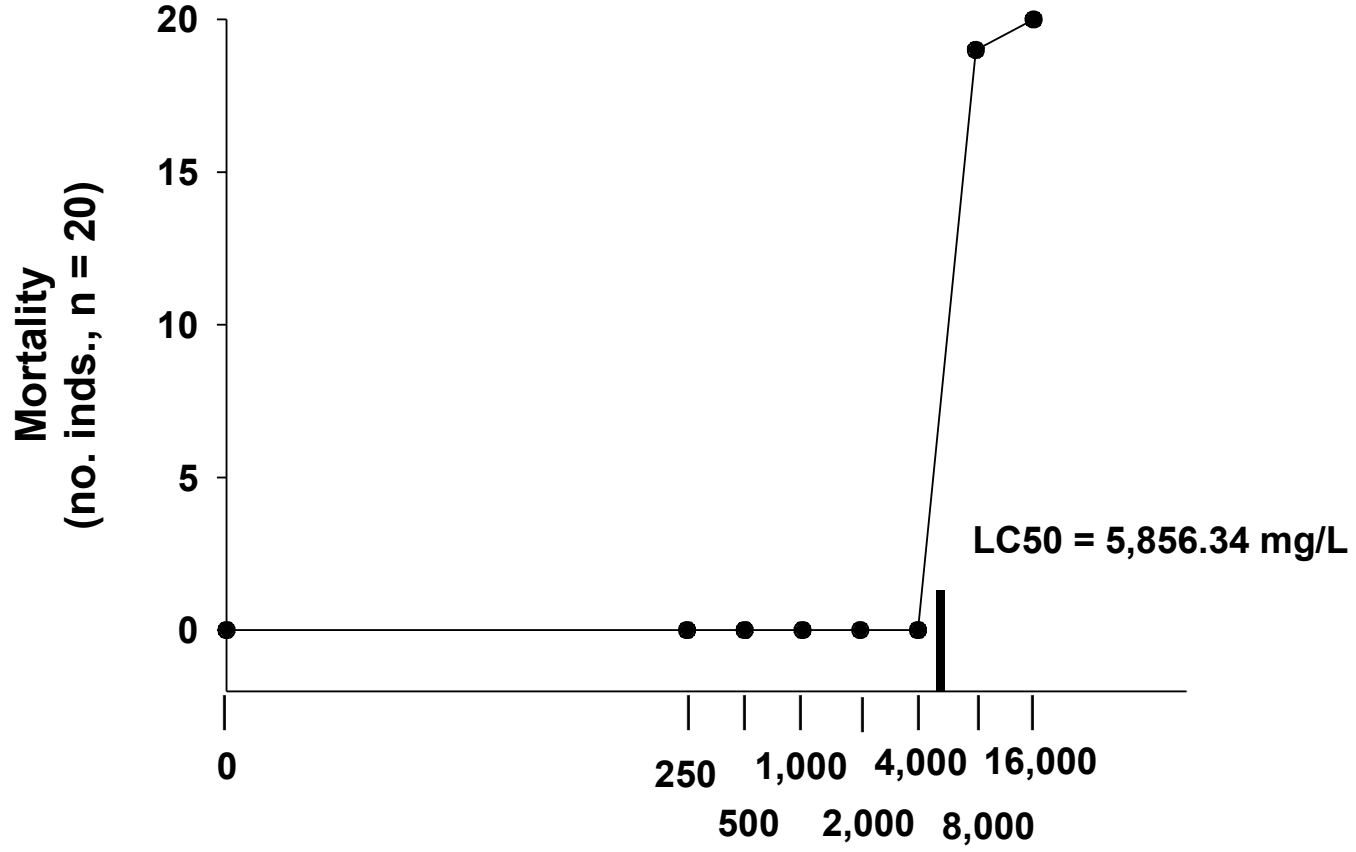
http://sepond.com/fish-stocking/fathead-minnows


Mortality of *Pimephales promelas*
(Y = 233.0 - 172.0X + 31.1X^2)
Percent Morality
(Angular Transform)
100
95
75
50
25
5
0
LC-50
100
1,000
10,000
100,000
OSE-II Concentration
(mg/L, log transform)

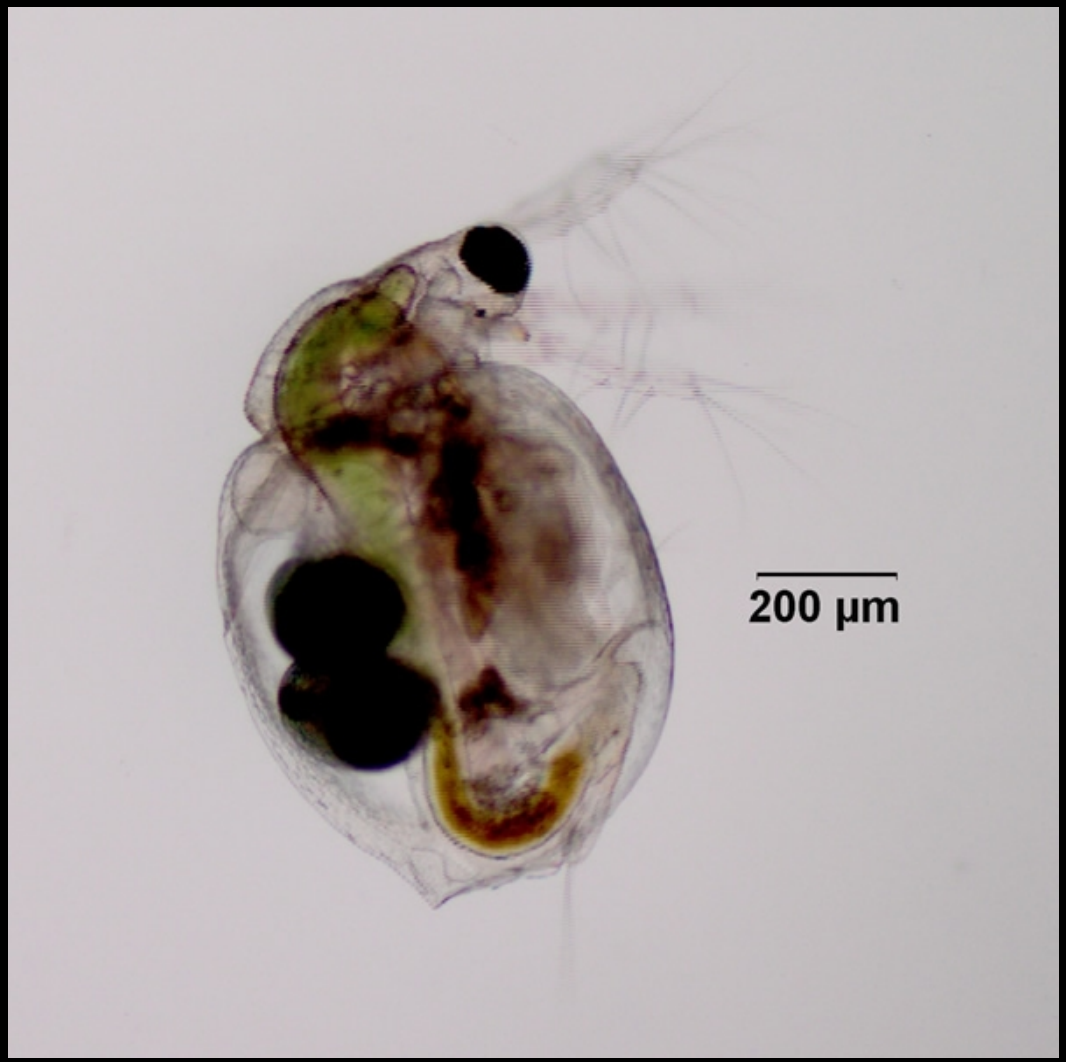
Enzymatic Bioremediation
(OSE-II)
Toxicity
***Pimephales promelas* (Fathead Minnow)**



Enzymatic Bioremediation
(OSE-II)
Toxicity - Mortality
Pimephales promelas (Fathead Minnow)



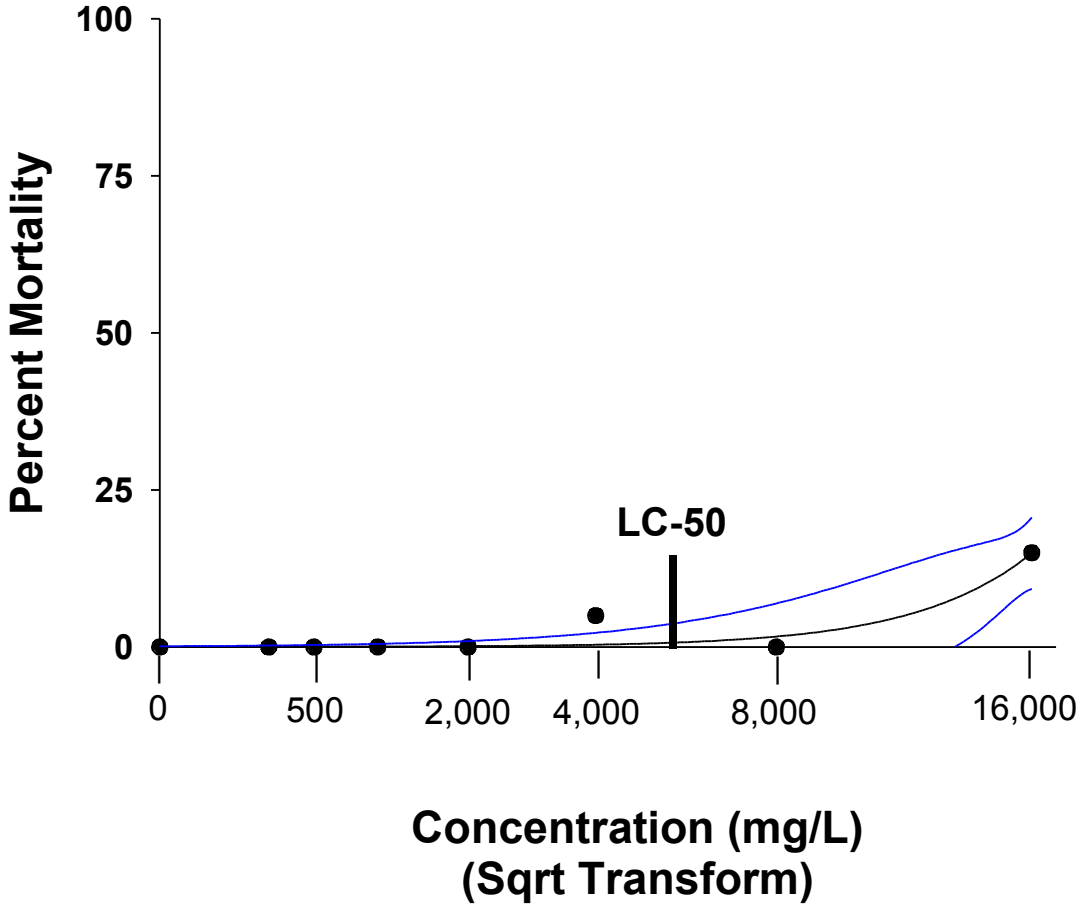
Ceriodaphnia dubia
(Freshwater water flea – planktonic)



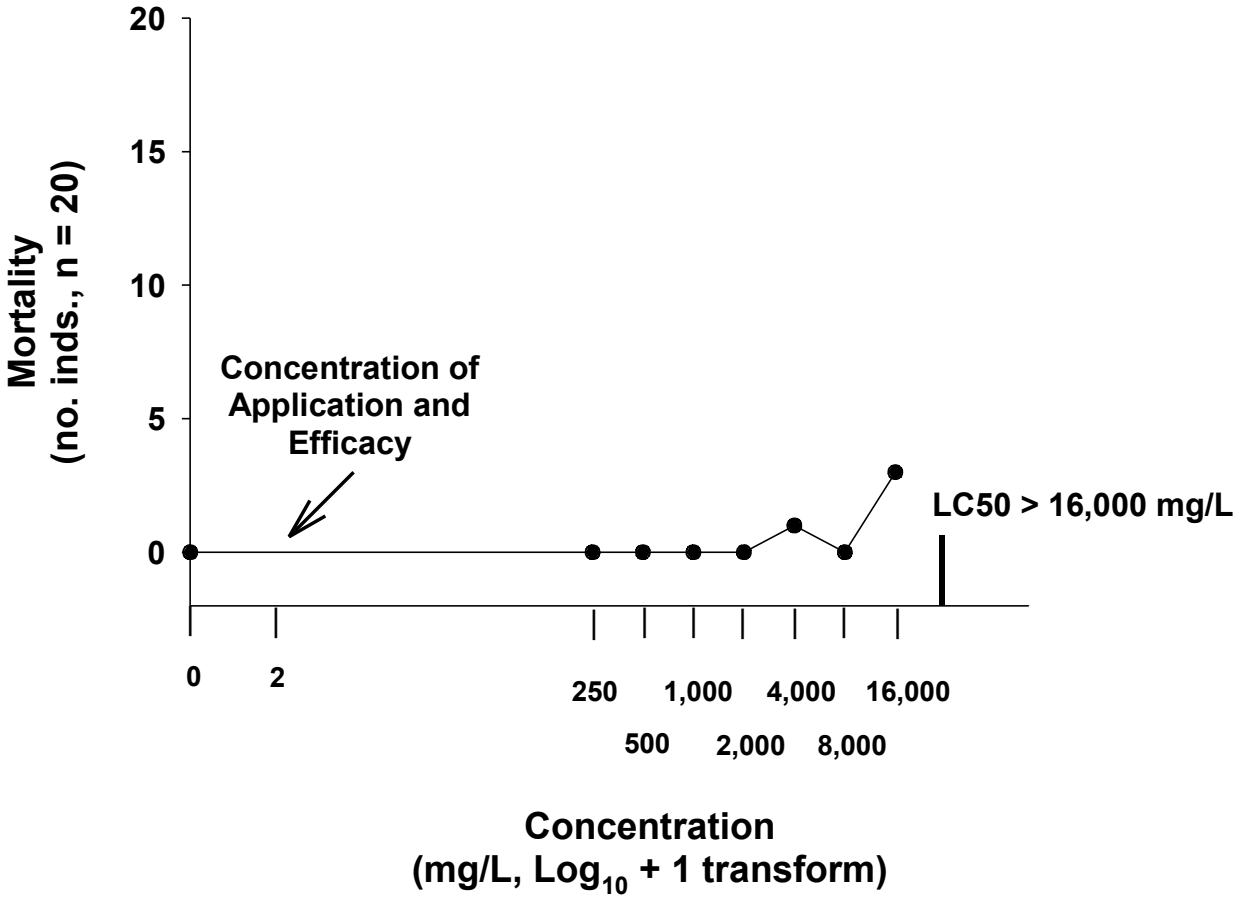
http://cfb.unh.edu/cfbkey/html/Organisms/CCladocera/FDaphnidae/GCeriodaphnia/Ceriodaphnia_dubia/ceriodaphniadubia.html

Enzymatic Bioremediation
Toxicity - *Ceriodaphnia dubia*
(planktonic crustacean)
(Using OSE-II)

$$Y = 37.3/(1+e^{(-(x-428.1)/16.8)})$$



Enzymatic Biotreatment
(OSE-II)
Toxicity - Mortality
Ceriodaphnia dubia (f.w. water flea)

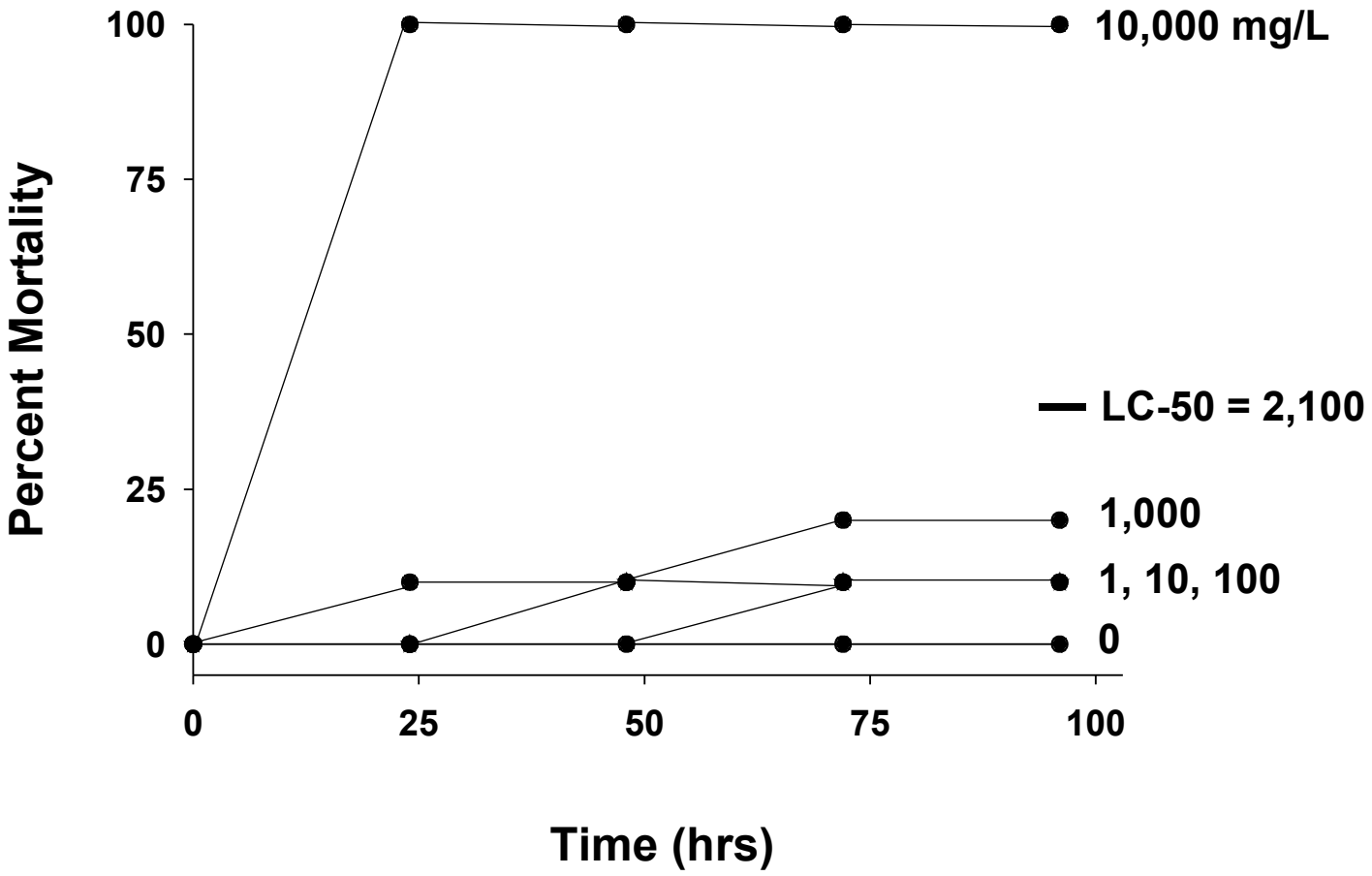


(Huther & Assocs., 2008)

Mysidopsis bahia
(Estuarine/marine mysid shrimp)

http://www.dailykos.com/story/2010/5/30/871254/-

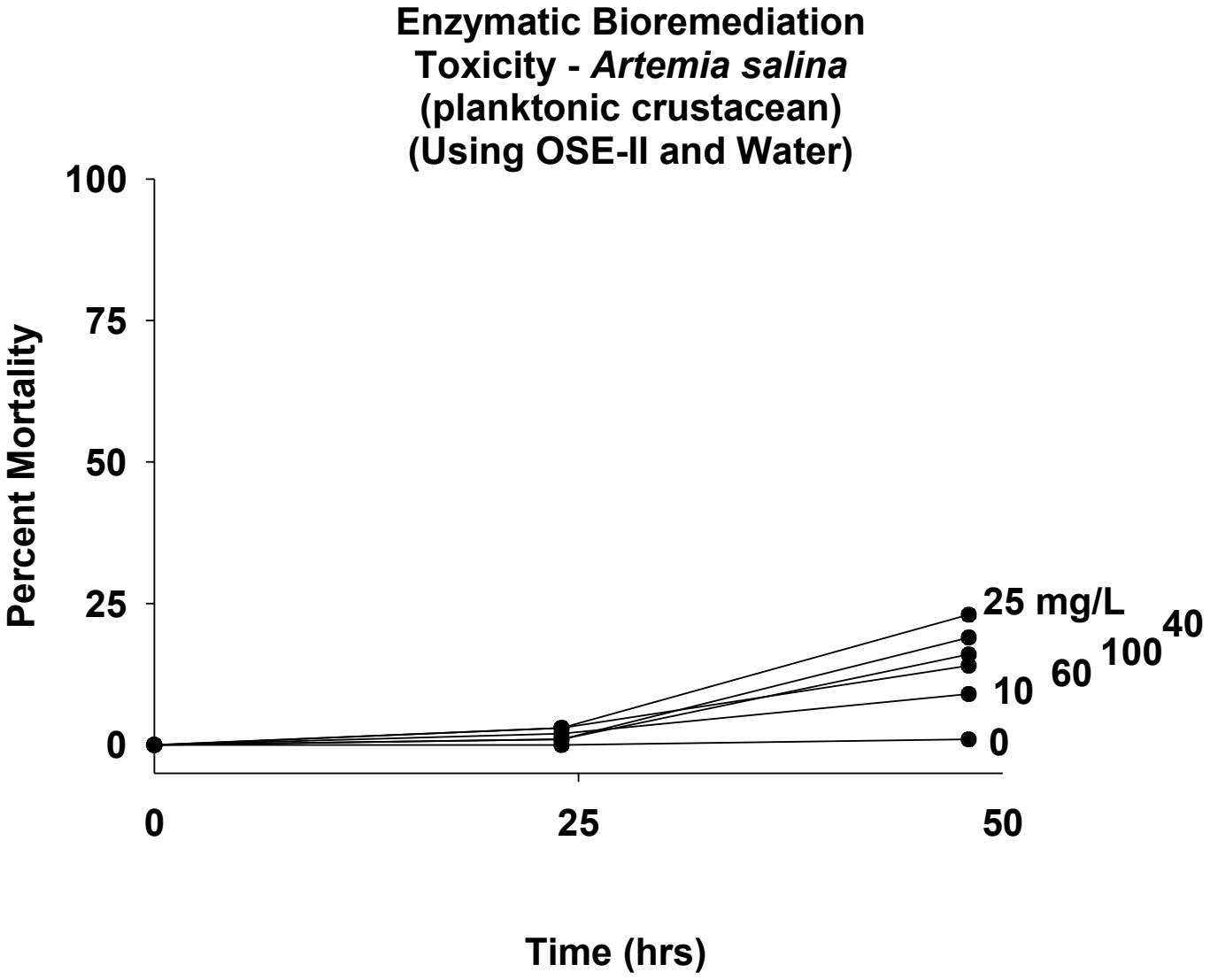
OSE-II
Enzymatic Bioremediation
Toxicity - *Mysidopsis bahia*
(mysid shrimp)



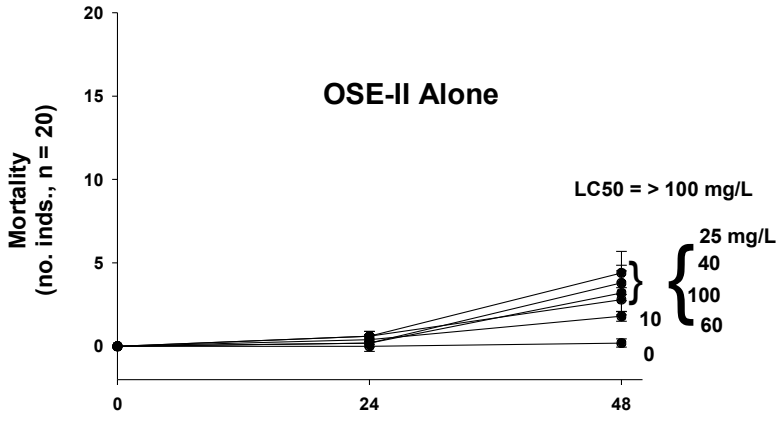
EnviroSystems Division
Resource Analysts, Inc.

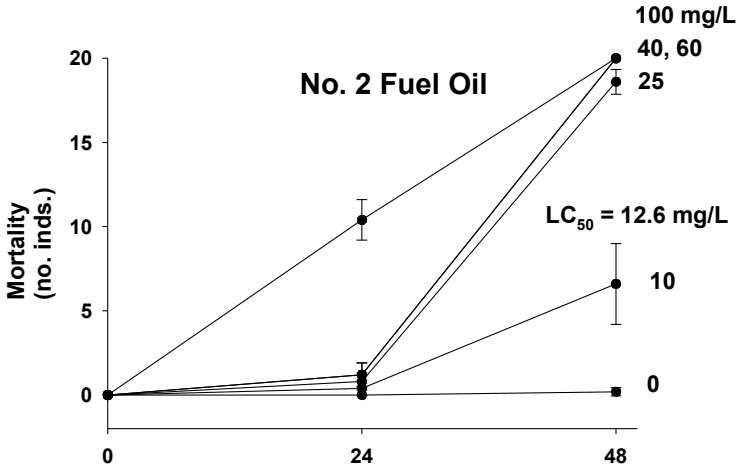
Artemia salina
(Freshwater brine shrimp, “Sea Monkeys”)

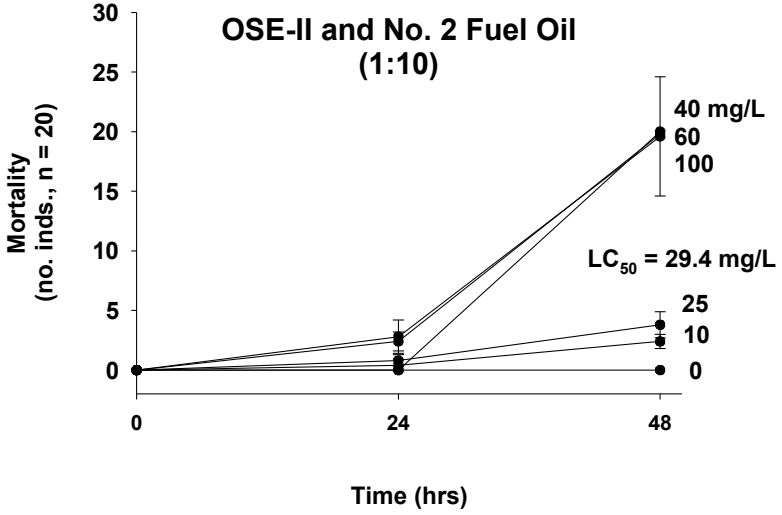
http://geneticsandbeyond.blogspot.com/2013/06/the-puffins-lair-to-be-pink-


Enzymatic Bioremediation
Toxicity - *Artemia salina*
(planktonic crustacean)
(Using OSE-II and Water)
Percent Mortality
100
75
50
25
0
0
25
50
Time (hrs)
25 mg/L
40
100
60
10
0

Enzyme Bioremediation (OSE-II) Toxicity Mortality in *Artemia salina* (freshwater plankton)







Additional Test Organisms

Oncorhyncus mykiss
(Pacific salmon)

Daphnia magna
(fw plankton)

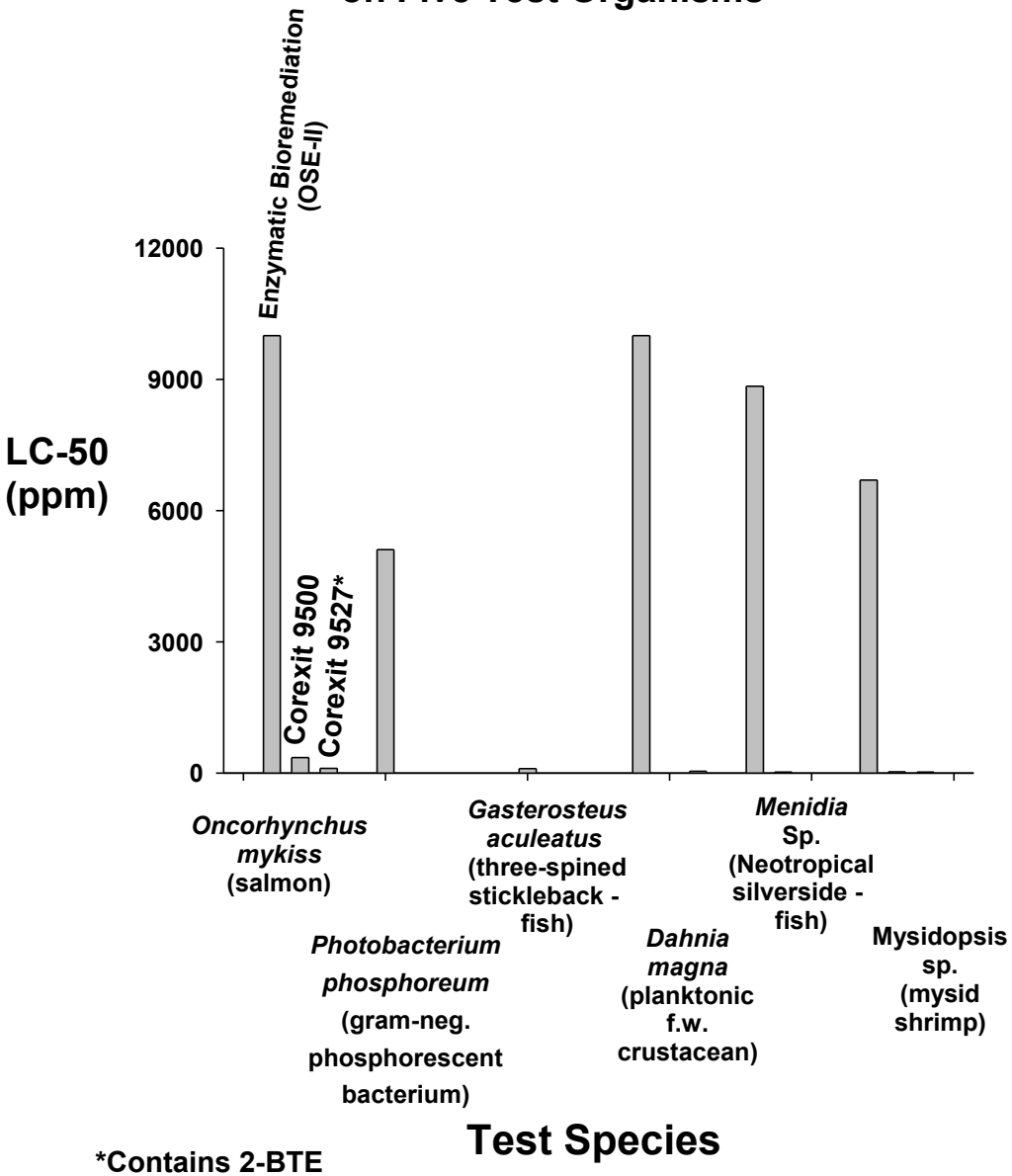
Menidia **sp.**
(Neotropical Silverside)

Photobacterium phosphorum
(gram-negative phosphorescent bacterium)

Gasterosteus aculeatus
(fw three-spined-stickleback)

Mysidopsis sp.
(estuarine/marine shrimp)

LC-50s for Two Corexit Products and OSE-II Enzymatic Bioremediation Product, on Five Test Organisms



*Contains 2-BTE

Toxicity to Humans

http://www.thesleuthjournal.com/whats-left-gulf-mexico/

STEVE COWPER, GOVERNOR

DEPARTMENT OF LABOR

OCCUPATIONAL SAFETY AND HEALTH
LABOR STANDARDS AND SAFETY DIVISION

(OSHA)

3301 EAGLE STREET, SUITE 303
P.O. BOX 107022
ANCHORAGE, ALASKA 99510-7022
PHONE: (907) 264-2597

August 23 1989

North Country Investment
2522 Arctic Blvd.
Anchorage, Alaska 99503

Corporate Office as of Oct. 1996:
OSEI, CORP.
13127 Chandler Drive
Dallas, Texas 75243

Attn: Steve Kacz

Dear Mr. Kacz:

An inquiry was made to this office concerning Sky Blue Chems "Oil Spill Eater." Specifically, we were asked to assess whether or not the use of this product would pose any health concerns by reason of the properties of the constituents.

Upon review of the material safety data sheet and other documents, we see no special toxicological concern with the ingredients that would pose a significant health concern with its application as described.

We would appreciate knowing in advance of any field tests or uses of this product.

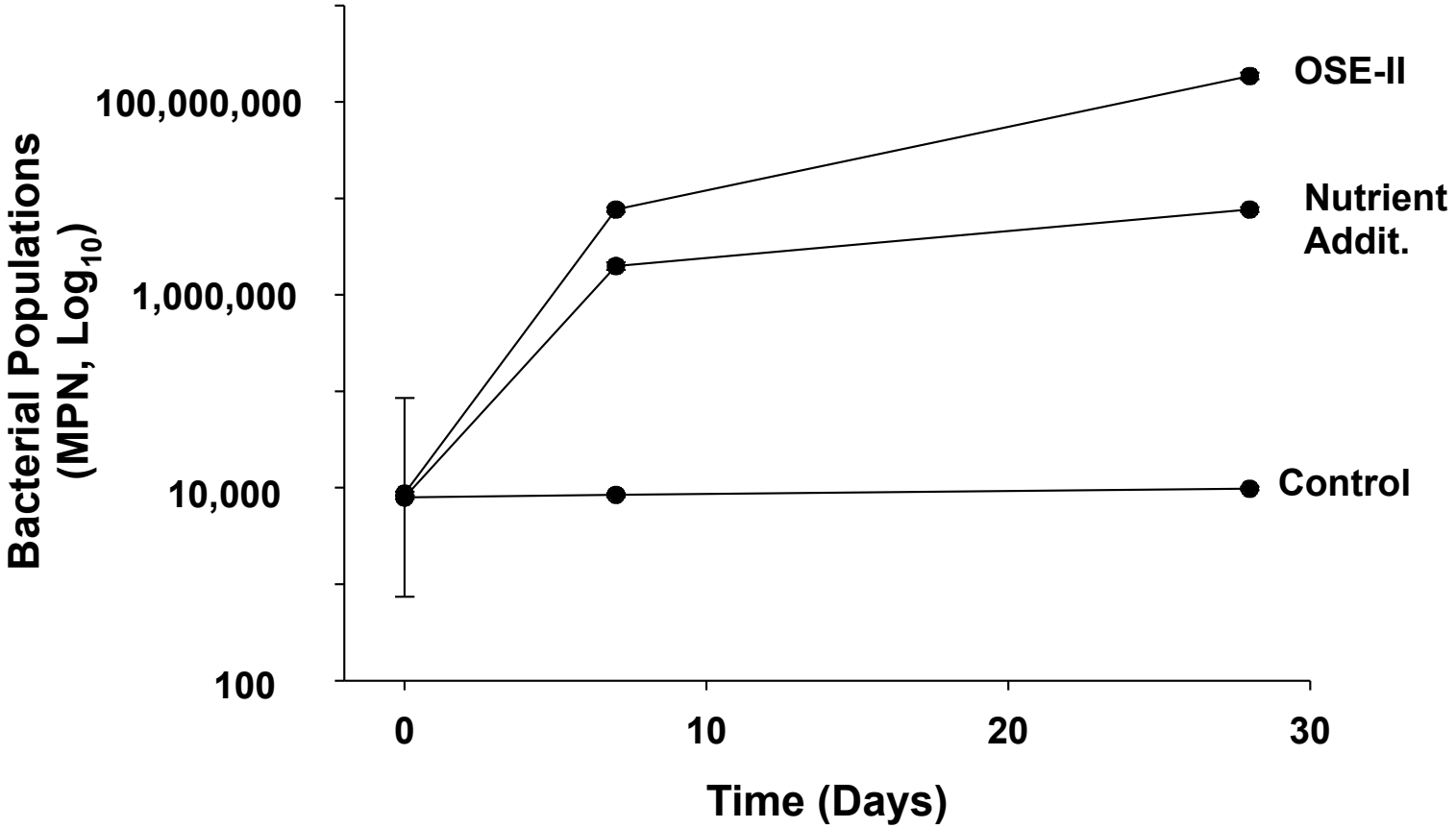
Sincerely,

Dennis L. Smythe
Chief of Compliance

cc: Ron Biggers

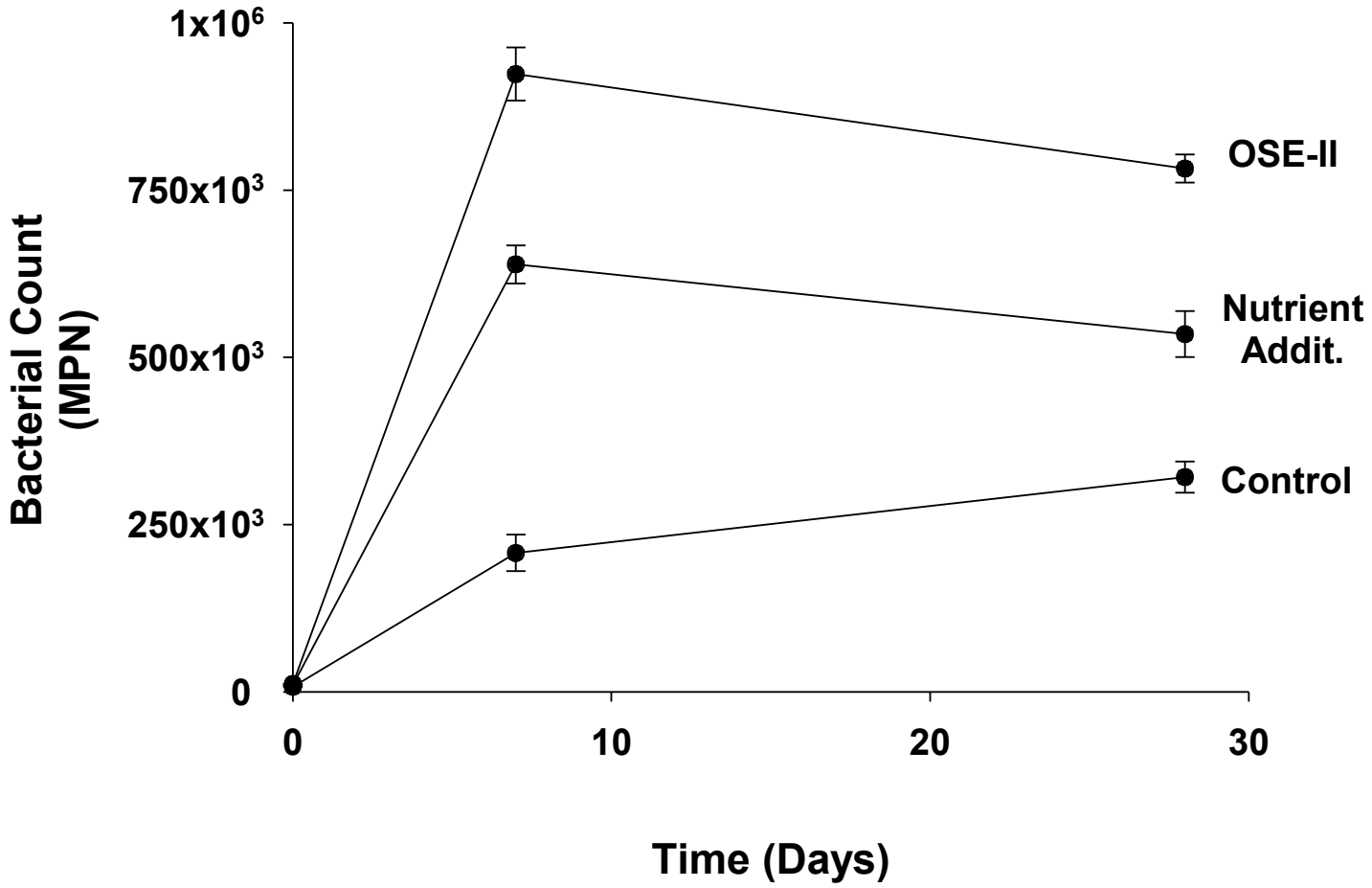
Bacterial Enhancement

Enzymatic Bioremediation (OSE-II) Bacterial Enhancement



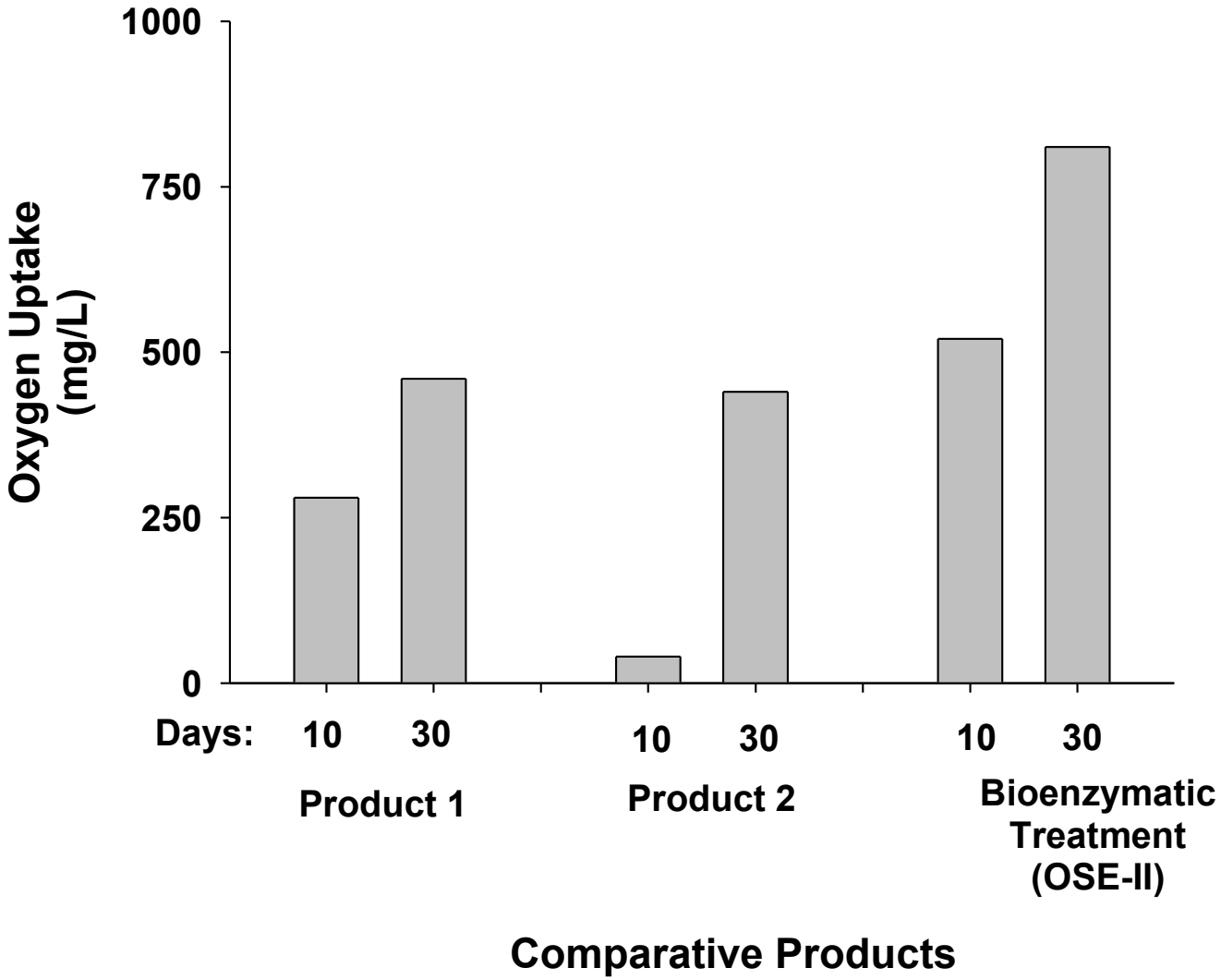
(Bio-Aquatic Testing, NELAC Certified)

Enzymatic Biotreatment (OSE-II) Bacterial Response



(U.S. Dept. Interior, BOEM; and Dept. Envtl. Sci., LA State Univ.)

Respirocity Test
Indicator of Oxygen-Enhanced Bacteria



Enhancement of Bacterial Population Growth on Crude Oil after application of Enzymatic Bioremediation treatment (OSE-II)

(OSE-II)

Cost Comparisons
BP/Deepwater Horizon Spill

Est. cost of treatment – in situ burning, mechanical recove chemical dispersants, damage claims, fines.

- **Est. spillage – 200M gallons**

- **Total est. cost of remediation - $42B**
- **Est. cost of clean-up = $210/gal oil**

Cost of alternate approach – Enzymatic Bioremediation

- **Total est. cost of remediation - $800M**
- **Est. cost of clean-up = $4/gal oil (Savings – 98.1%)**

Countries using Enzymatic Bioremediation on Oil Spills with Governmental Approval

- Australia
- Bahrain
- Colombia
- Greece
- Iraq
- Iran
- Kenya
- Kuwait
- Mexico
- New Zealand
- Nigeria
- Oman
- Philippines
- Qatar
- Saudi Arabia
- South Korea
- Trinidad and Tobago
- United Arab Emirates
- United Kingdom
- United States (Military - Army, Navy, Coast Guard Air Force, Marines)

Acknowledgements

The Lawrence Anthony Earth Organization (LAEO)

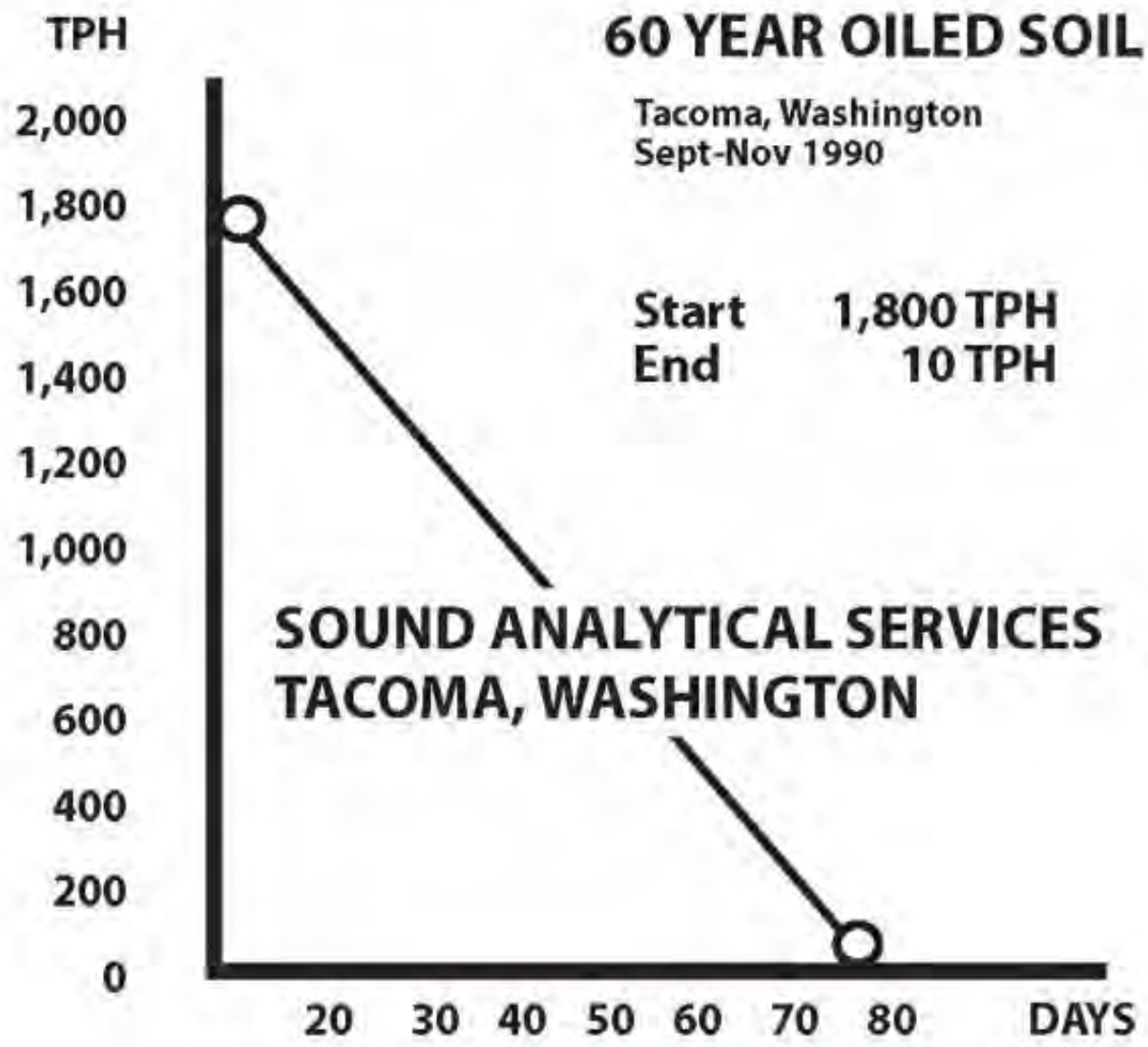
- **Ms. Diane Wagenbrenner**
- **Ms. Elizabeth Montgomery**
- **Ms. Barbara Wiseman**

Mr. Steven Pedigo, The OSE-II Corporation

Louisiana Universities Marine Consortium (LUMCON)

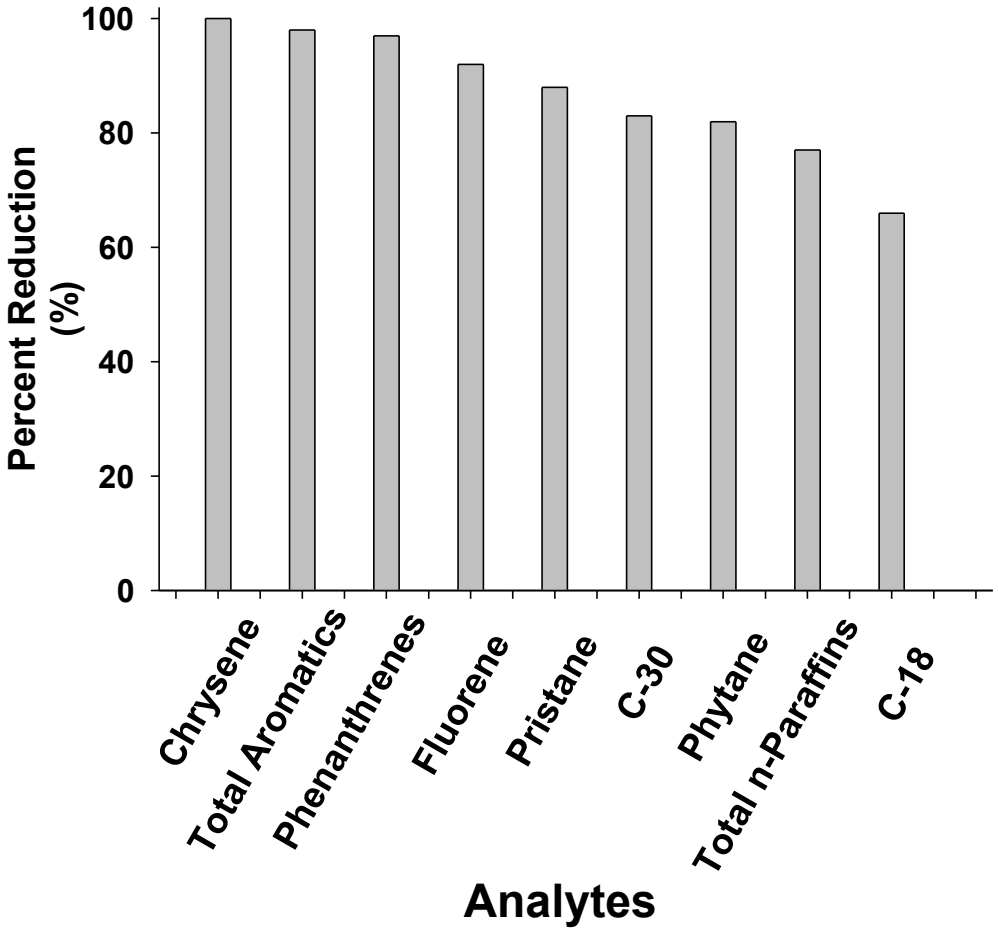
The 2016 Clean Gulf Conference – Ms. Cassie Davie

	Dispersants	Mechanical	Enzymatic Bioremediation
Clean-up	0%	2-8%	~ 100%
Toxicity	Toxic (To marine spp. & humans, particularly w. 2-butoxy-ethanol - 2-BTE	Toxic (derived from oil)	Non-toxic (detoxifies oil quickly)


TPH
60 YEAR OILED SOIL
Tacoma, Washington
Sept-Nov 1990
2,000
1,800
1,600
1,400
1,200
1,000
800
600
400
200
0
Start 1,800 TPH
End 10 TPH
SOUND ANALYTICAL SERVICES
TACOMA, WASHINGTON
20 30 40 50 60 70 80 DAYS

Tier II Efficacy Data
Enymatic Bioremediation Treatment
(OSE-II)

Percent Reduction after 21 Days



OSE-II Toxicity Test - LC-50 (mg/ L)

Menidia beryllina and *M. bahia*

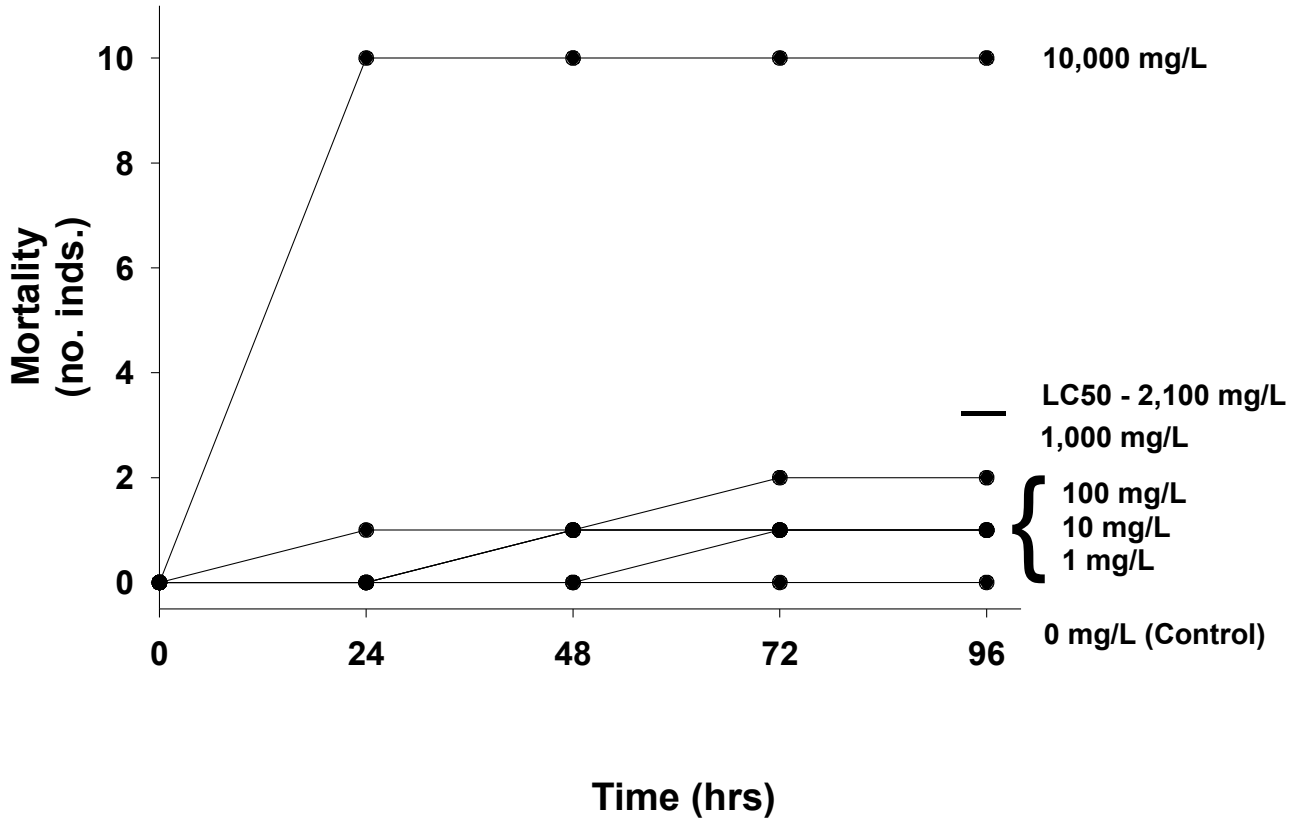
(Silversides - fish)

Species	Duration (hrs)		
	48	96	168
Menidia beryllina	*6698	5970	
	**5700	5700	2500
Menidia bahia	*8839	8839	

*Static

**Static renewal

Enzymatic Biotreatment
(OSE-II)
Juvenile Mysid Shrimp
Toxicity - Survivorship



(EPA/NETAC)

Mechanism of Action (cont.)

- Neutralizes toxicity to soil and aquatic or marine organisms
- Enzymes separate oil molecules and enhance their metabolic breakdown

Additional Advantages of Enzymatic Bioremediation

- No specific precautions need be taken
- No vapors emitted; thus, no inhalation problems
- A one-time application is sufficient to achieve effectiveness