

Enzymatic Bioremediation of Petroleum Hydrocarbons



November 1-3, 2016
Tampa Convention Center | Tampa, FL



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Characteristics of Enzymatic Biotreatment of Oil Spills

- . Biodegrades crude oil rapidly into smaller components and finally into CO₂ and H₂O
- . 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

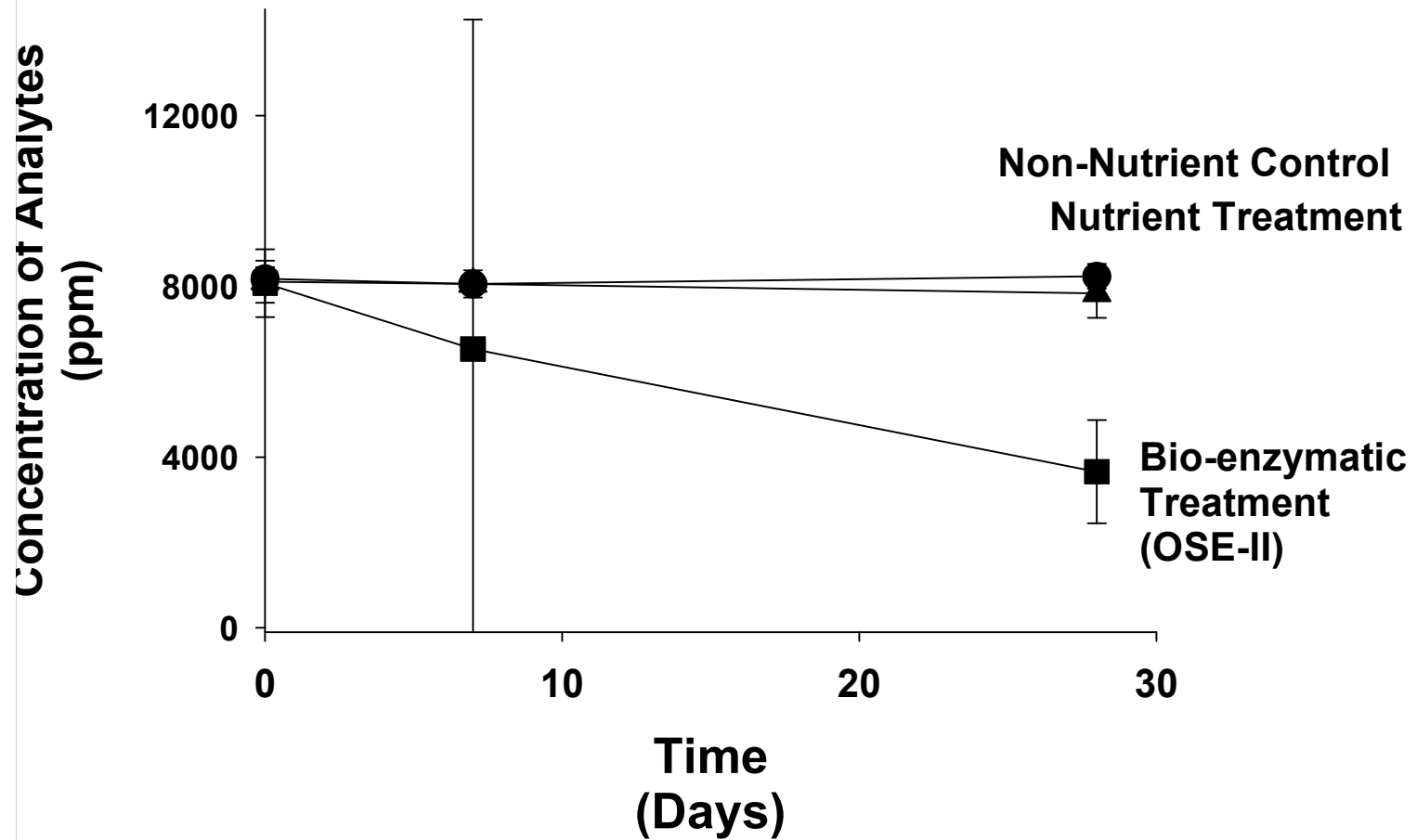
- . Contains
 - Bio-surfactants
 - Enzymes
 - Nutrients

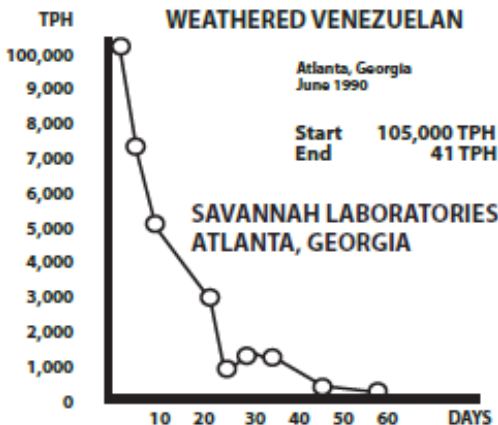
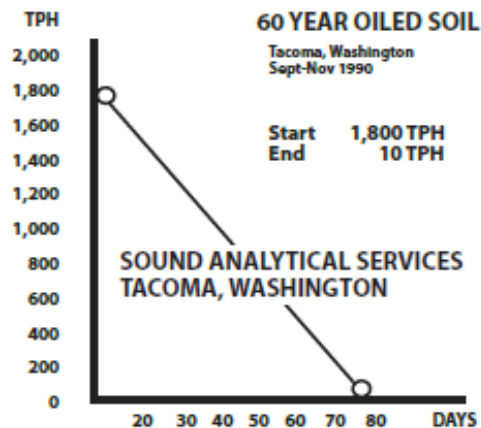
Testing

- . 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

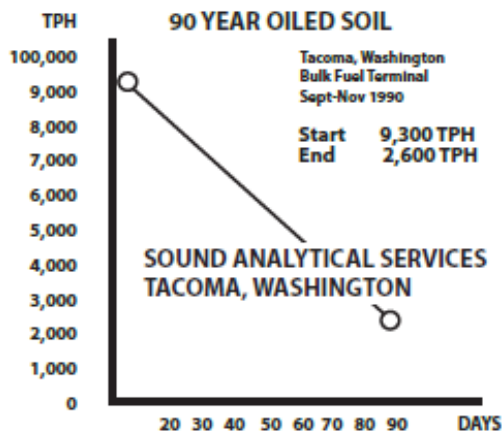
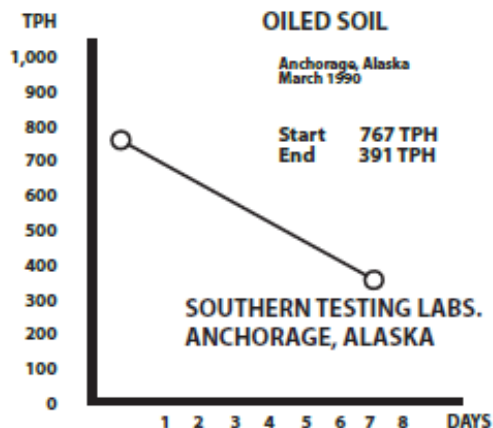
Efficacy

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





TPH Biodegradation Tests

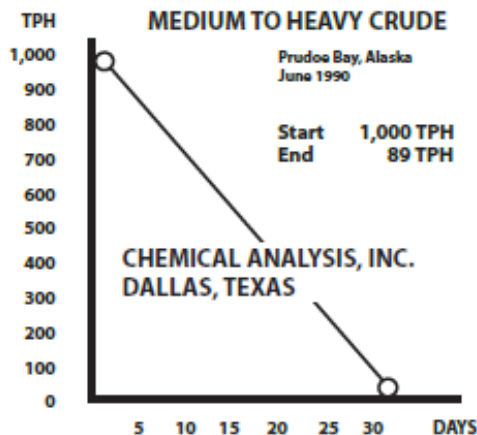
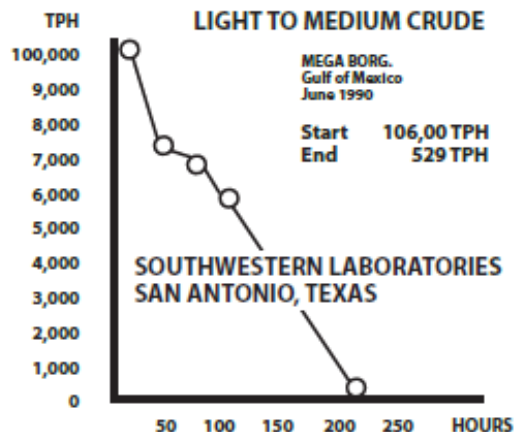


Using Enzymatic
Treatment of Crude Oil

Performed by

Independent Labs

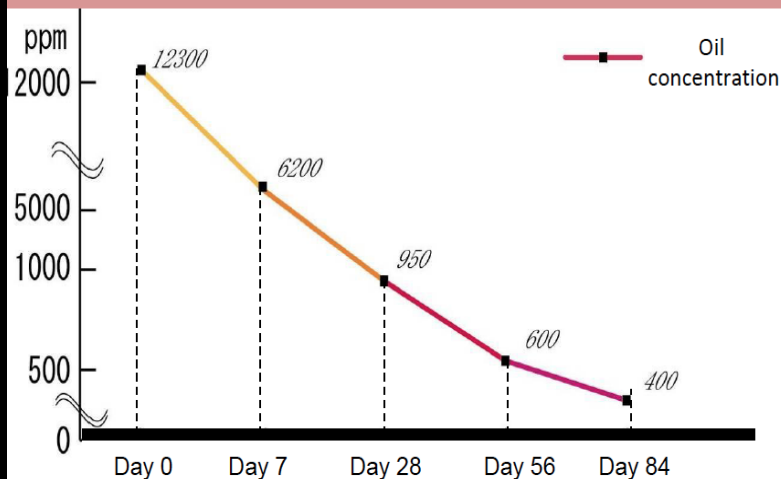
(OSE-II)



Data : Oil decompose rate of OSEII bio stimulation

Analysis By University of Yamanashi (JAPAN)

Proceedings oil decompose



About ppm (parts per million) :

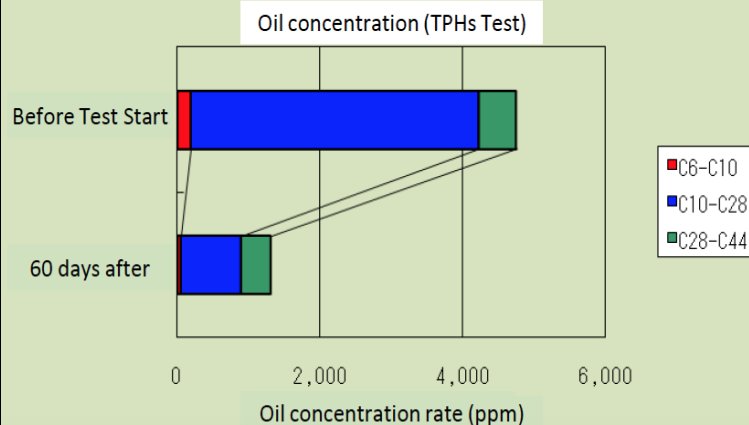
ppm is rate of contamination level of each soil.

We multiply amount of soil by ppm.

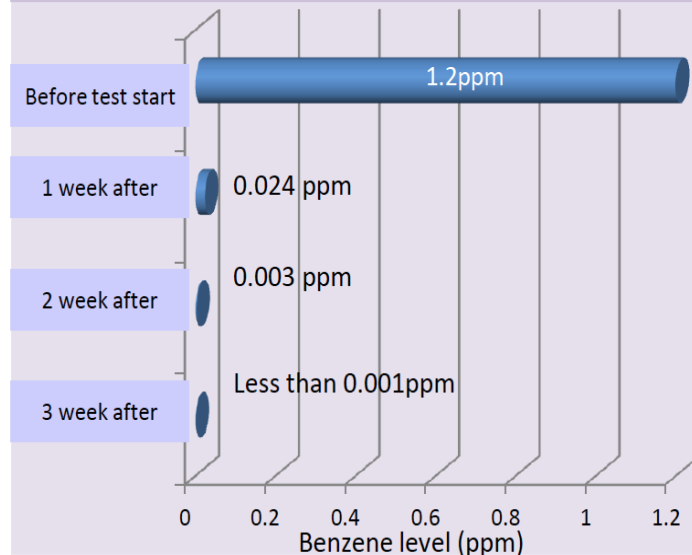
For Example: If the rate is 12300ppm from amount of 3000kg soil which means contain oil about 37kg (1.23%).

*Usually most of pollution rate is 1000-7000ppm.

Proceedings each oil decompose



Proceedings Benzene level

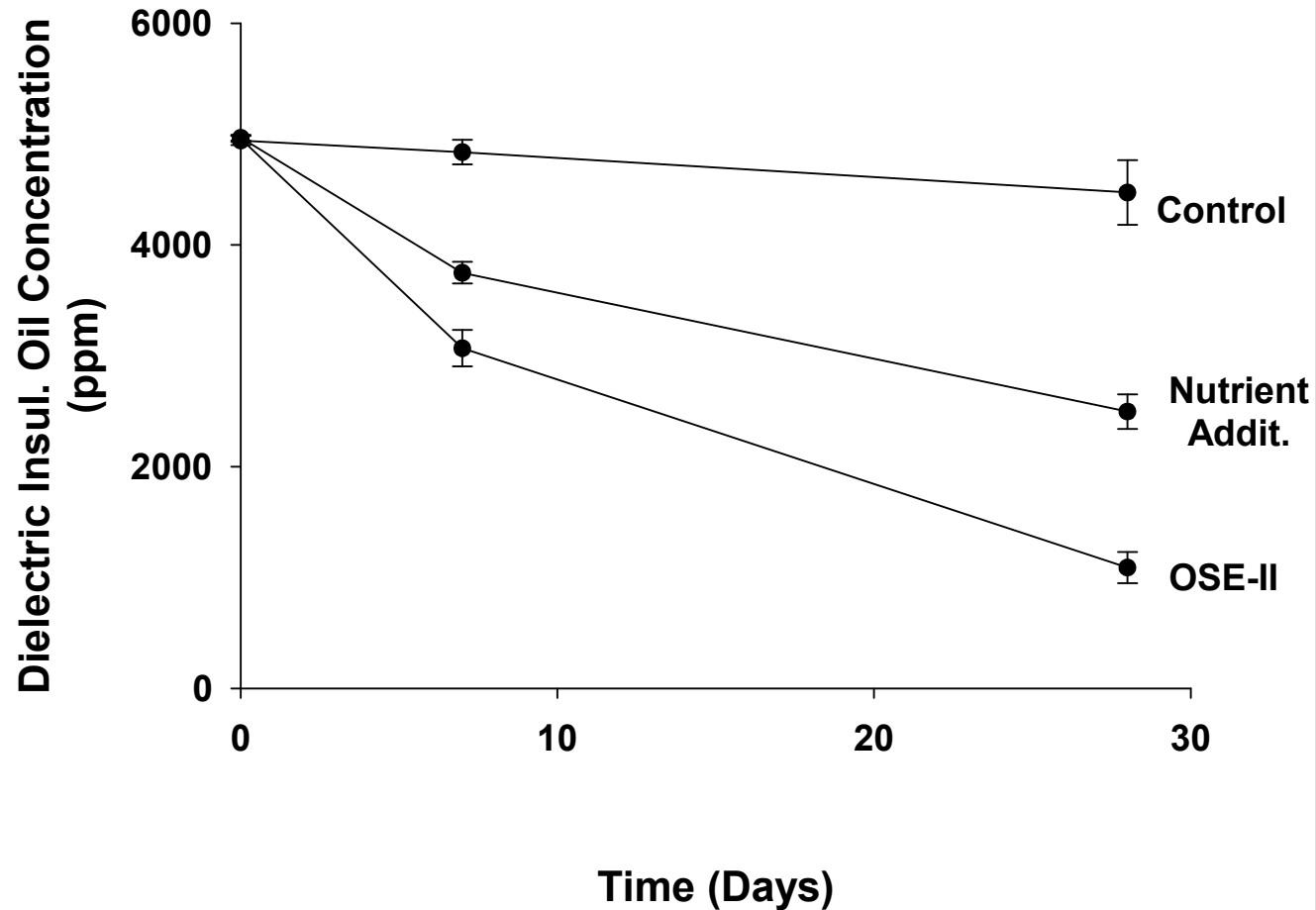


TPH = Total Petroleum Hydrocarbon

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**

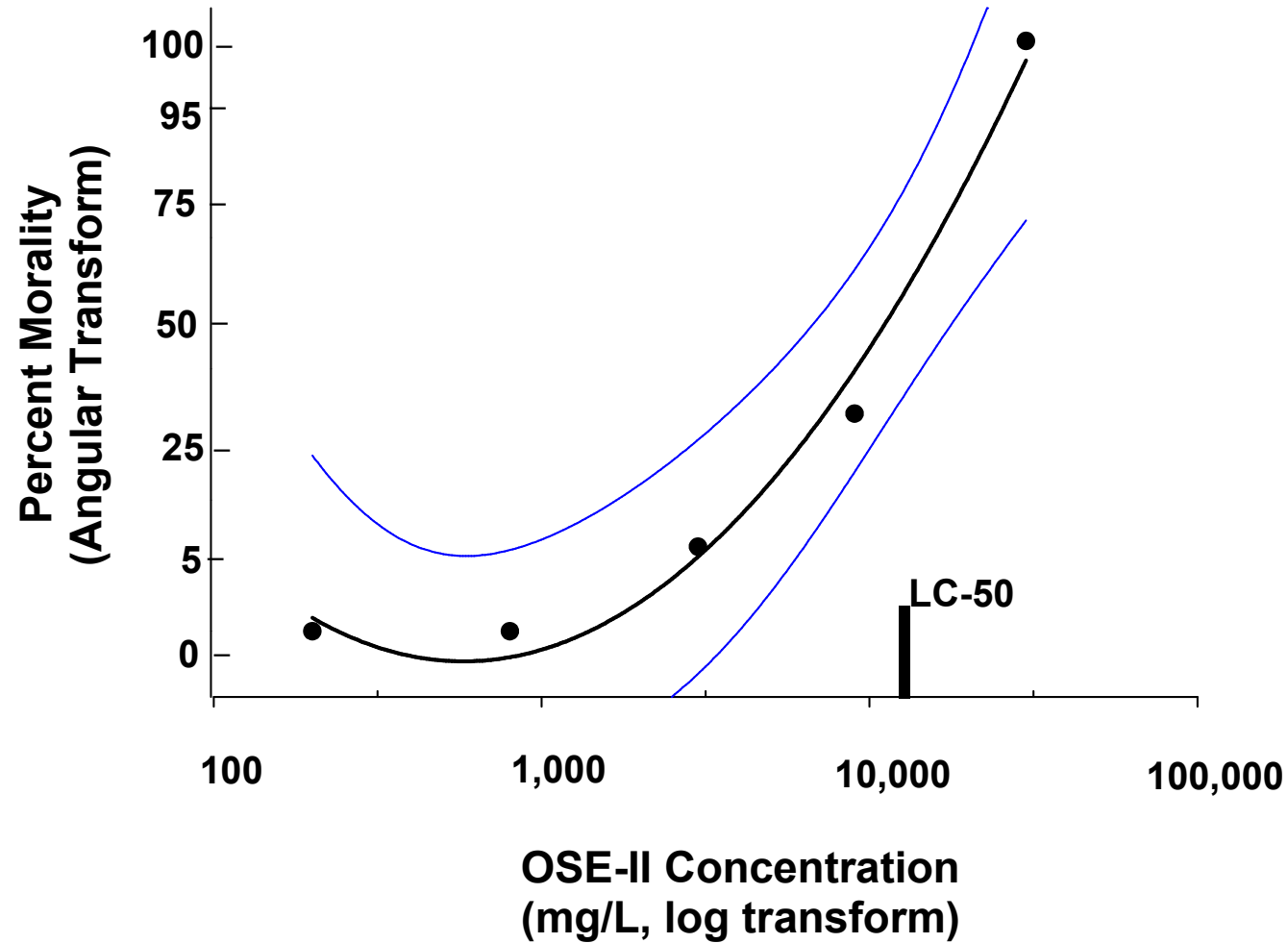


Toxicity

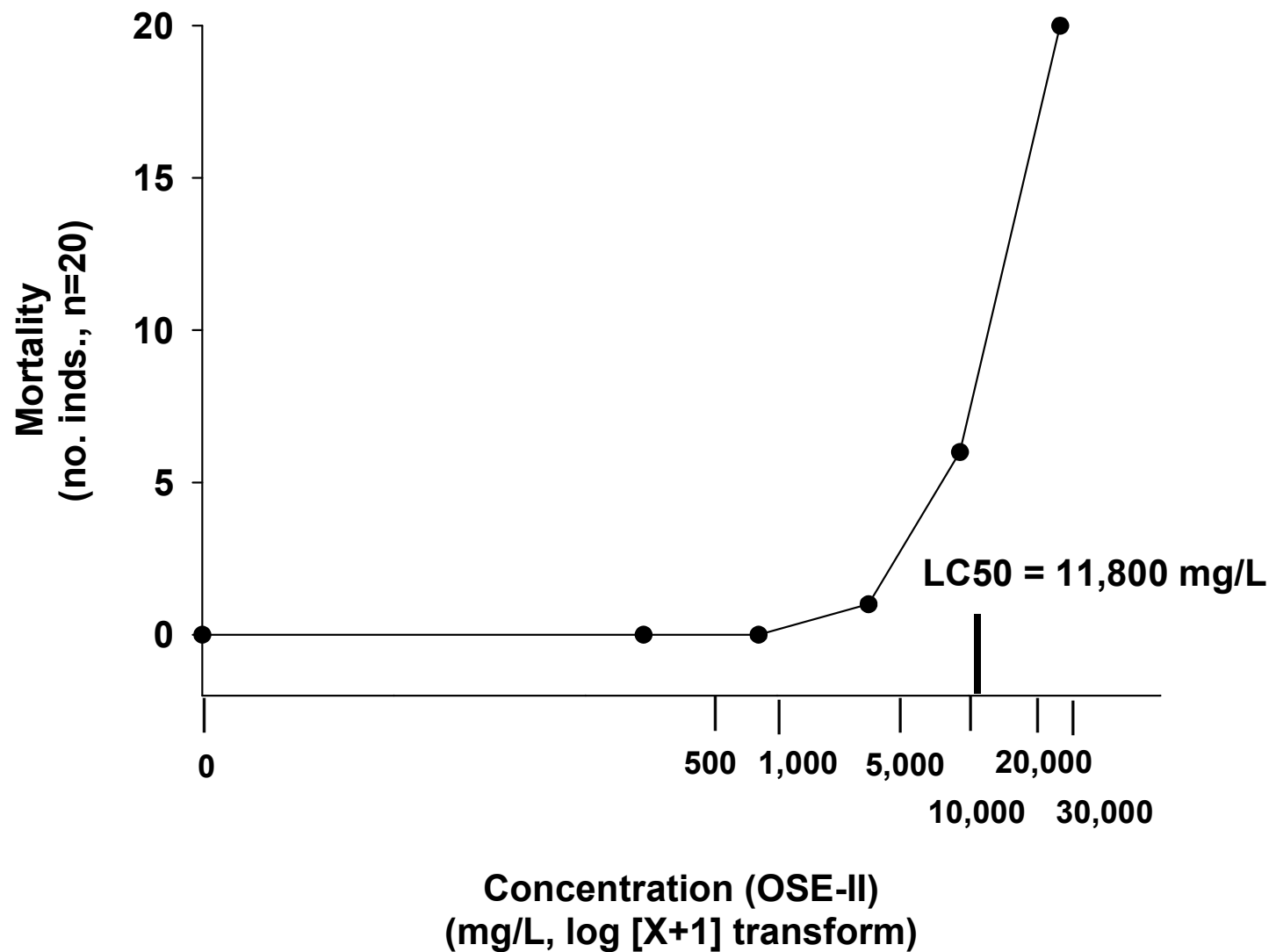
Pimephales promelas
(Fathead Minnow)



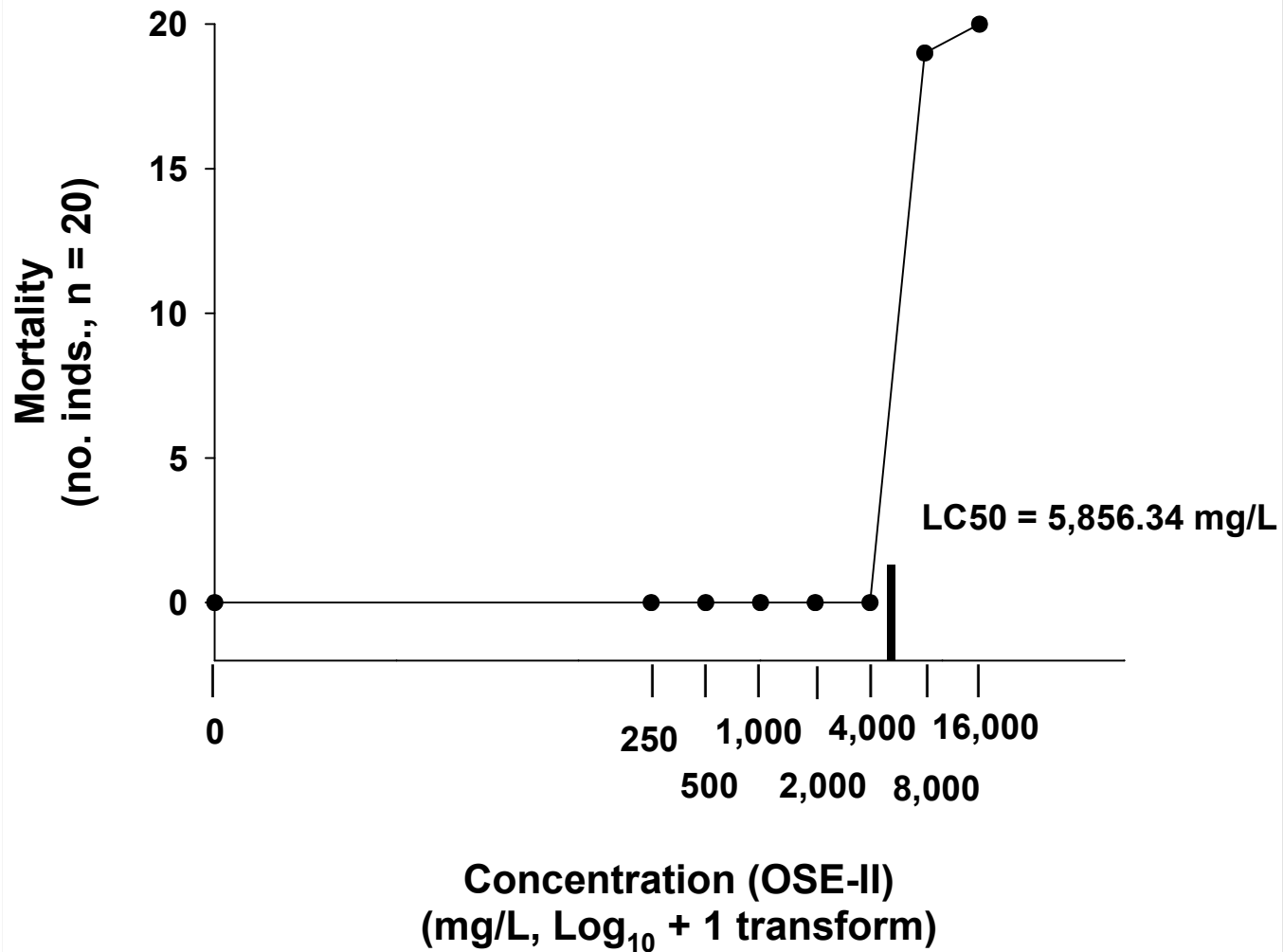
Mortality of *Pimephales promelas*
($Y = 233.0 - 172.0X + 31.1X^2$)



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



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

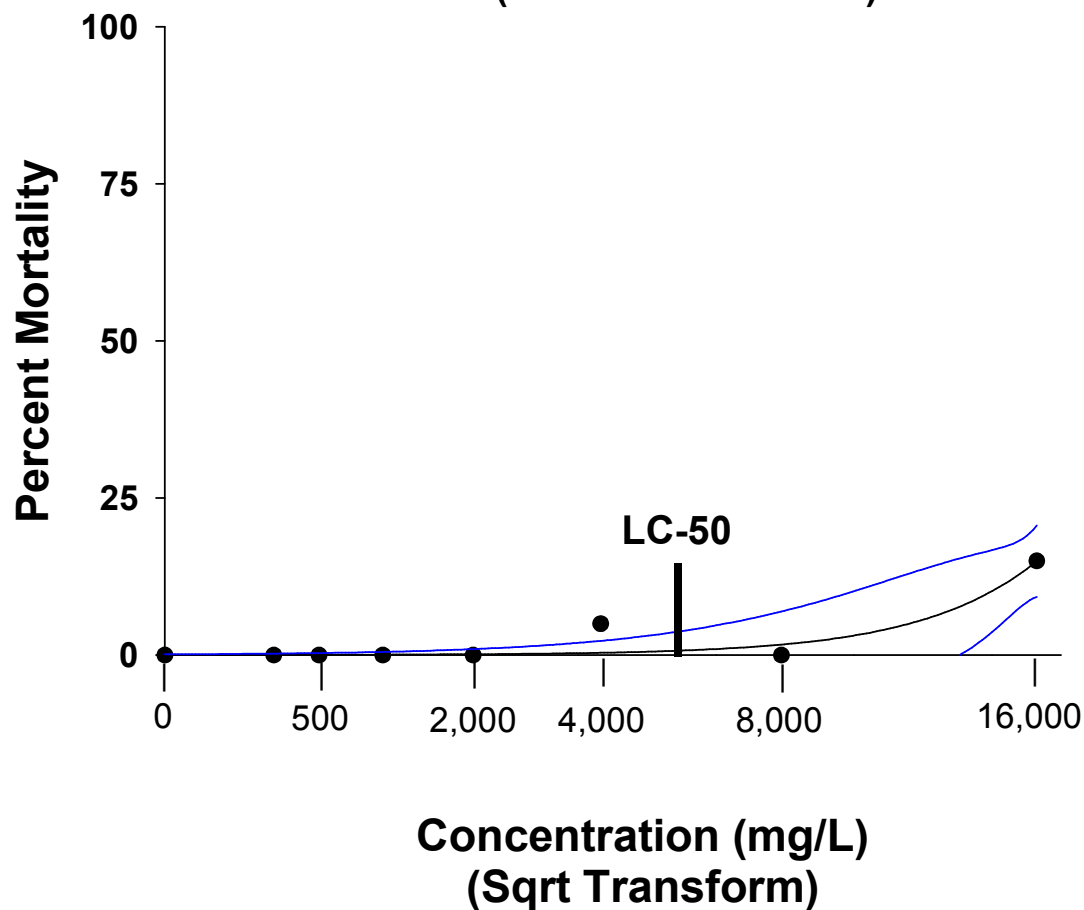


Ceriodaphnia dubia
(Freshwater water flea – planktonic)

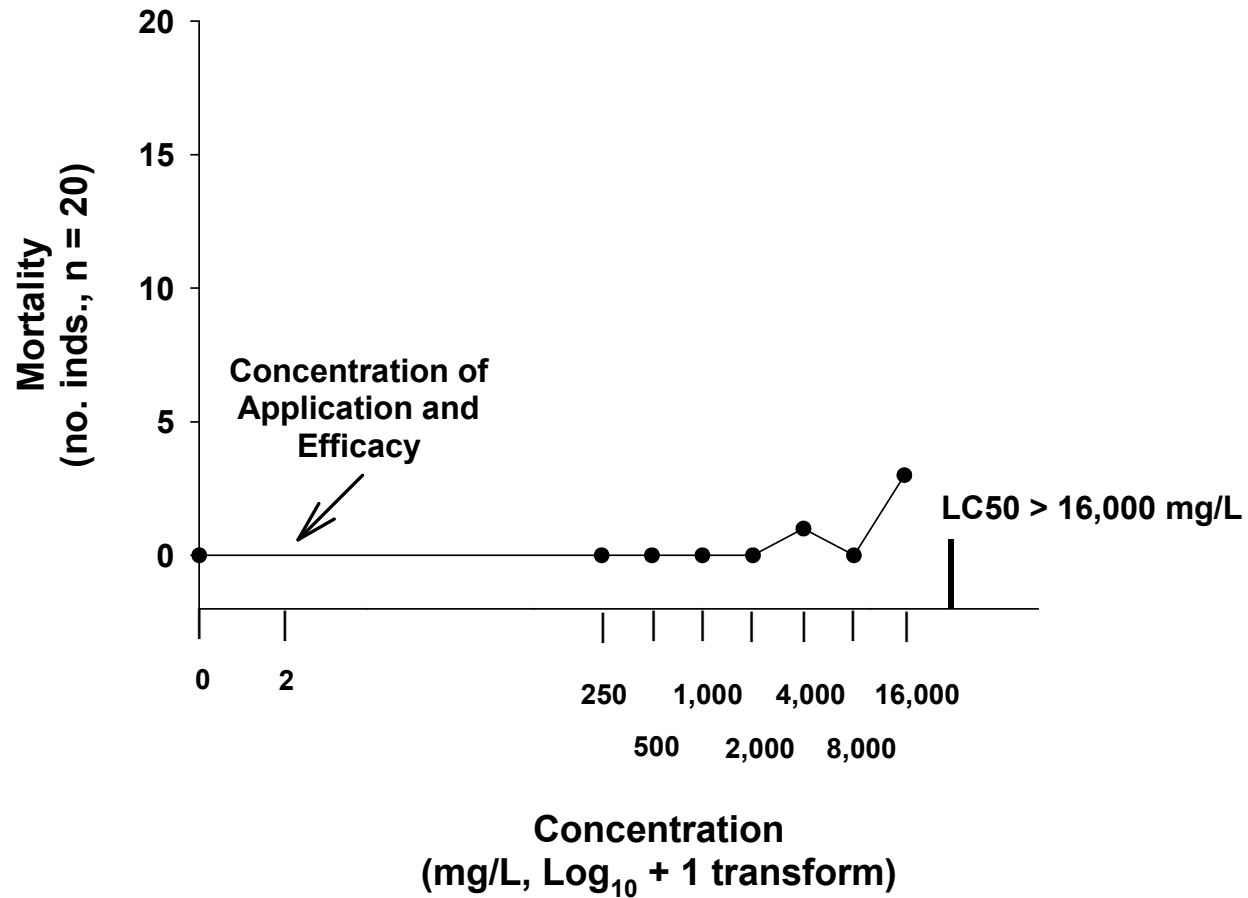


Enzymatic Bioremediation
Toxicity - *Ceriodaphnia dubia*
(planktonic crustacean)
(Using OSE-II)

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



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

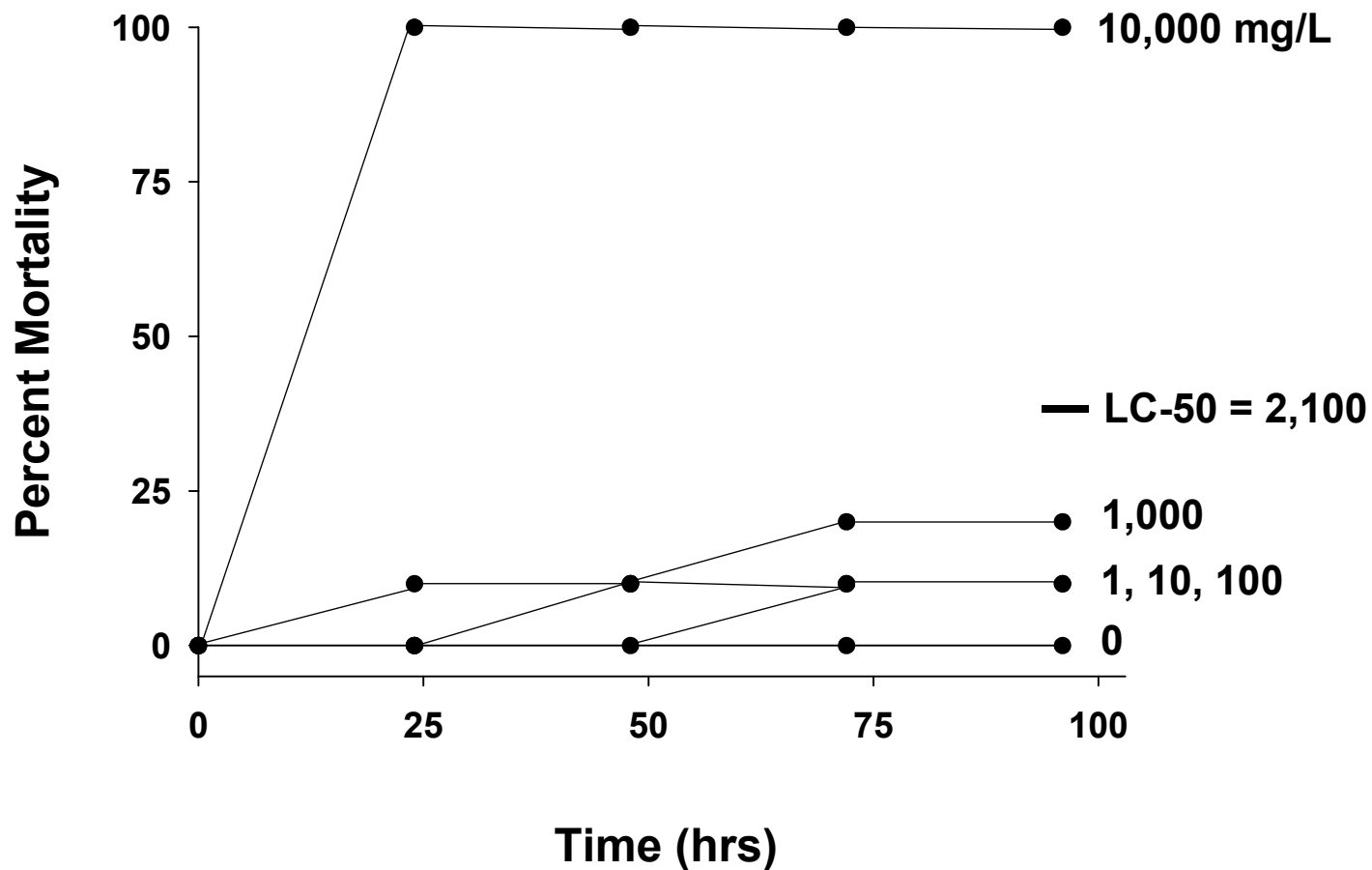


(Huther & Assocs., 2008)

Mysidopsis bahia
(Estuarine/marine mysid shrimp)



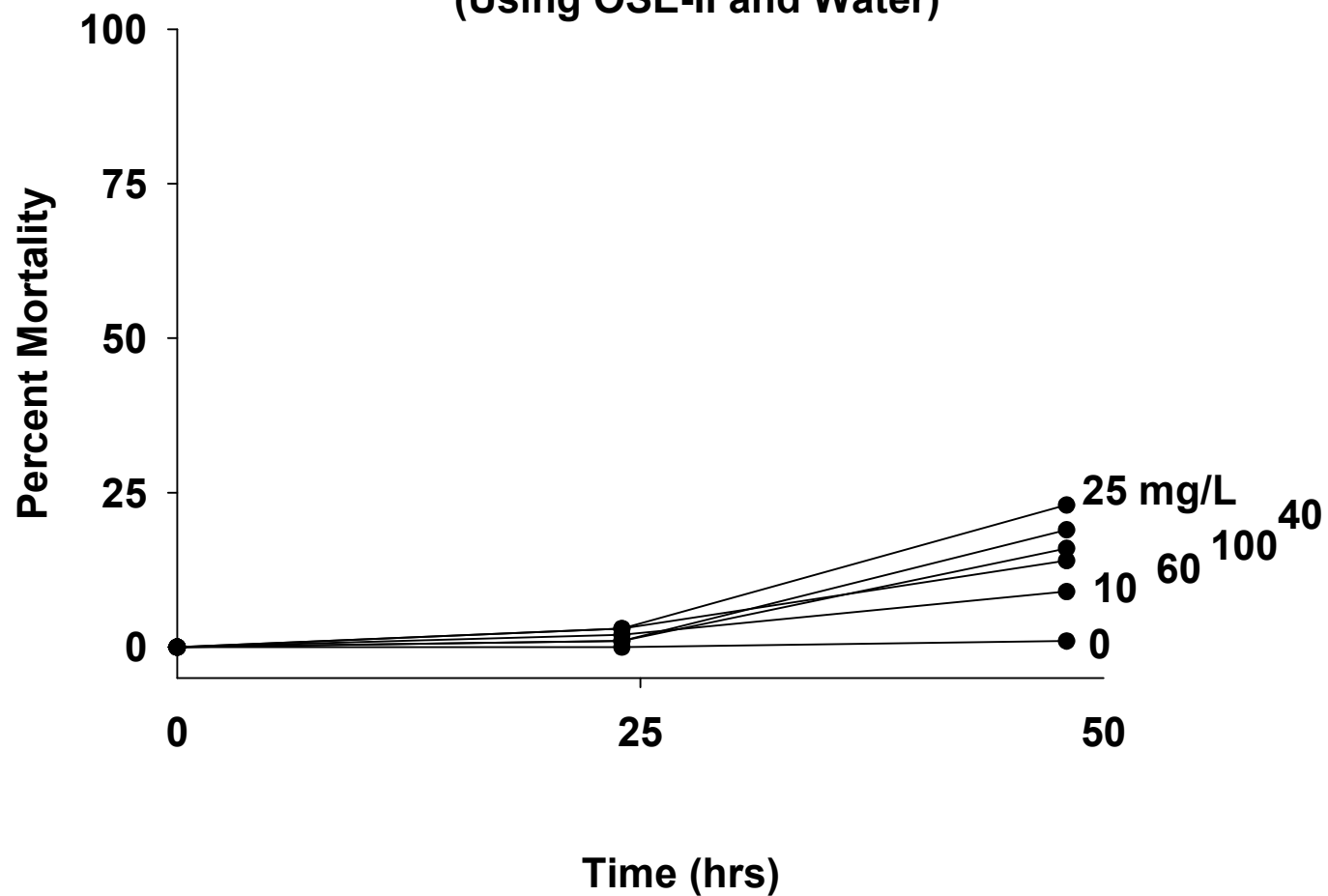
OSE-II
Enzymatic Bioremediation
Toxicity - *Mysidopsis bahia*
(mysid shrimp)



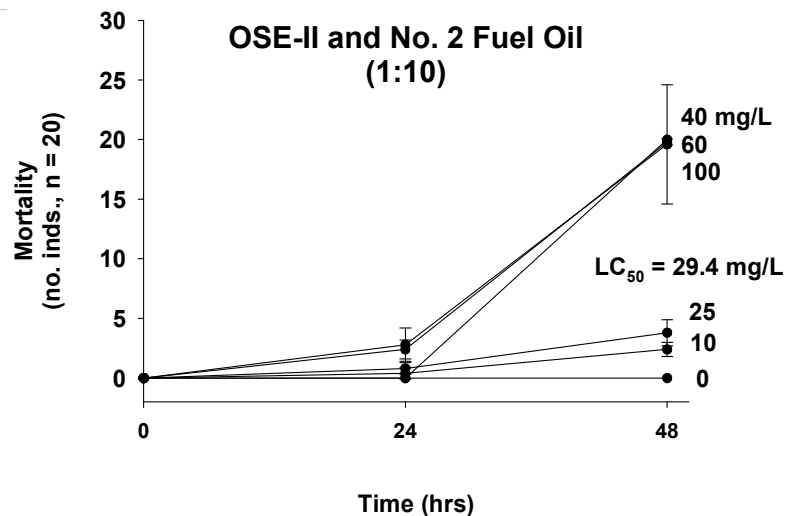
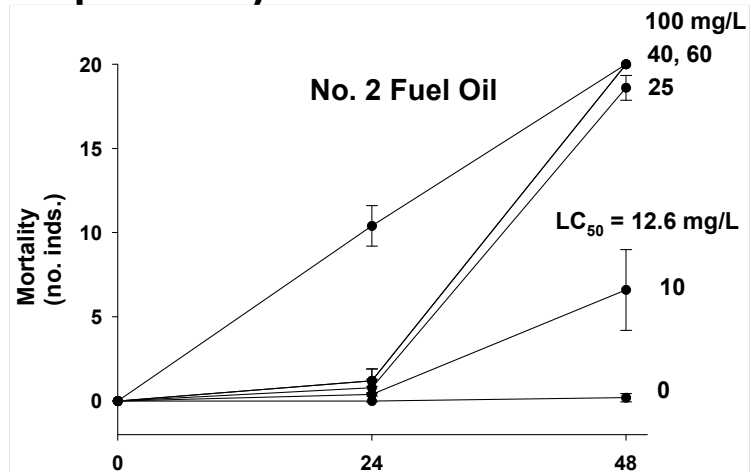
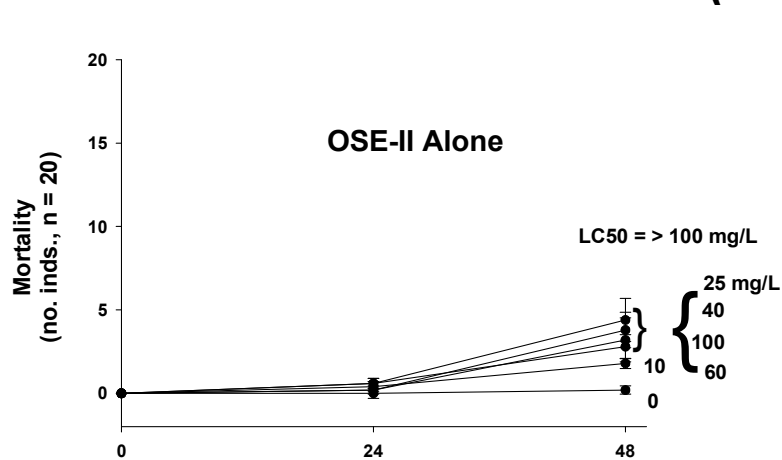
Artemia salina
(Freshwater brine shrimp, “Sea Monkeys”)



**Enzymatic Bioremediation
Toxicity - *Artemia salina*
(planktonic crustacean)
(Using OSE-II and Water)**



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



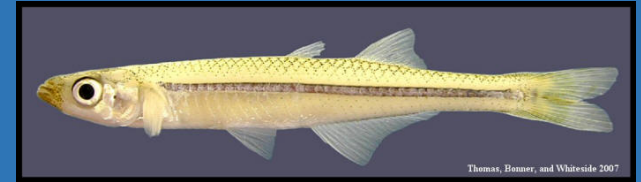
Additional Test Organisms



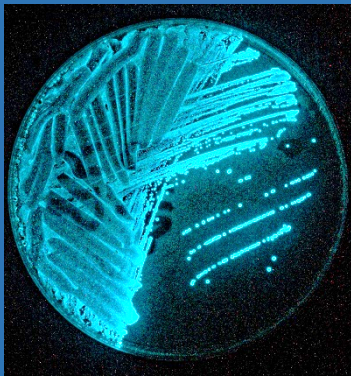
Oncorhynchus mykiss
(Pacific salmon)



Daphnia magna
(fw plankton)



Menidia sp.
(Neotropical Silverside)



Photobacterium phosphorum
(gram-negative phosphorescent
bacterium)

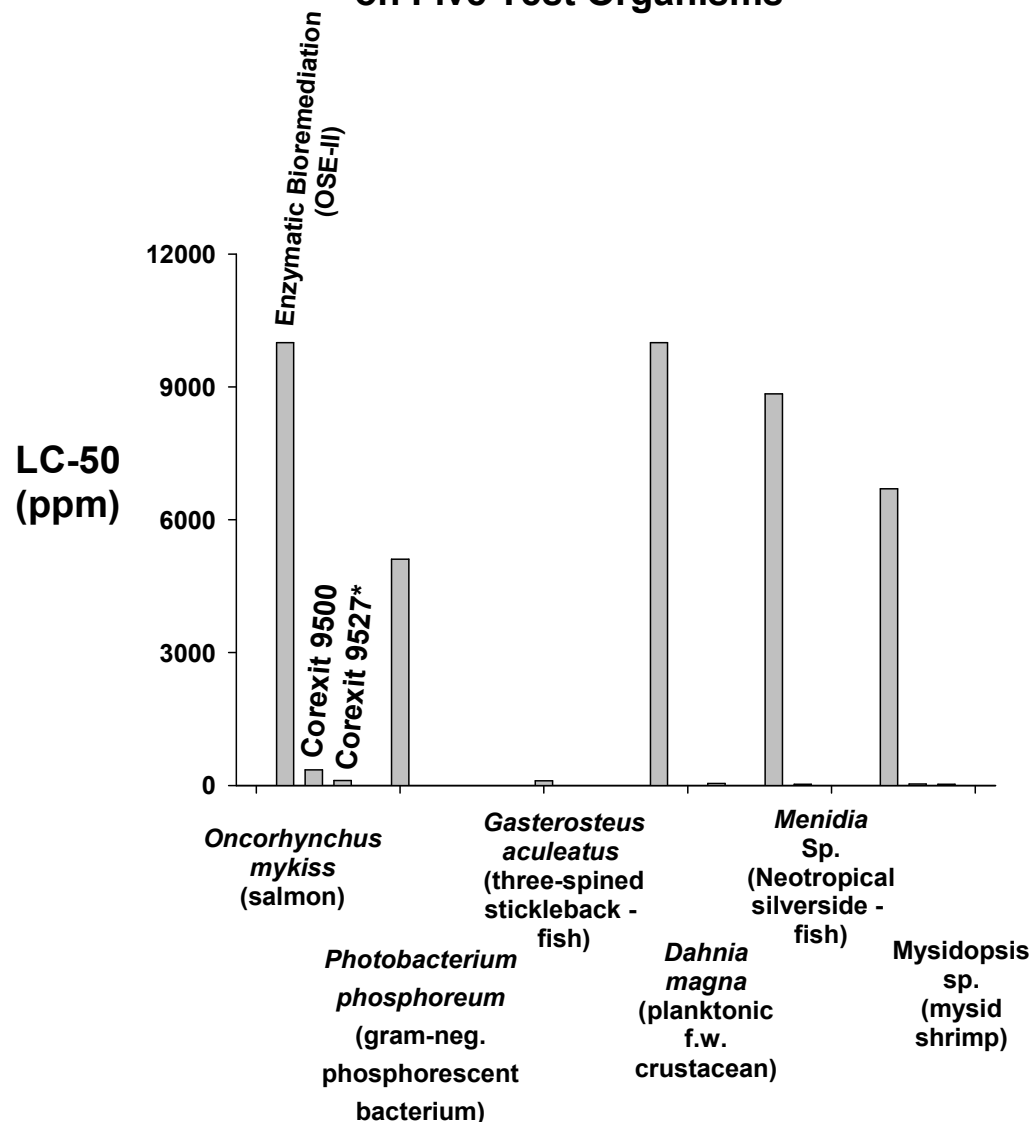


Gasterosteus aculeatus
(fw three-spined-stickleback)



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





STEVE COWPER, GOVERNOR

DEPARTMENT OF LABOR

**OCCUPATIONAL SAFETY AND HEALTH
LABOR STANDARDS AND SAFETY DIVISION**

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P.O. BOX 107022
ANCHORAGE, ALASKA 99510-7022
PHONE: (907) 264-2597

(OSHA)

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

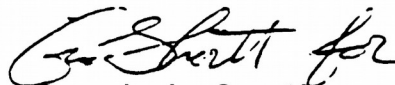
Dear Mr. Kacz:

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,

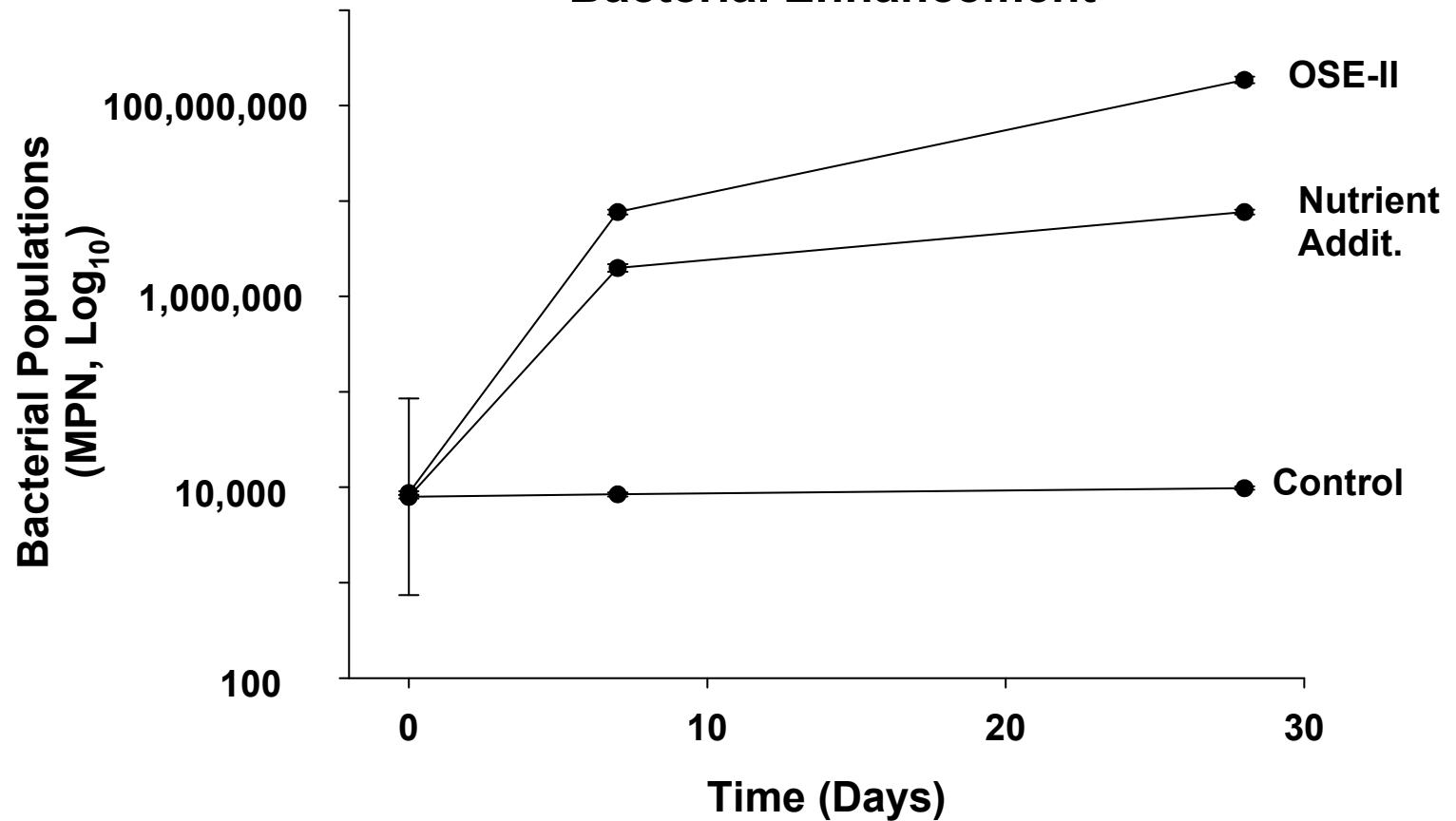


**Dennis L. Smythe
Chief of Compliance**

cc: Ron Biggers

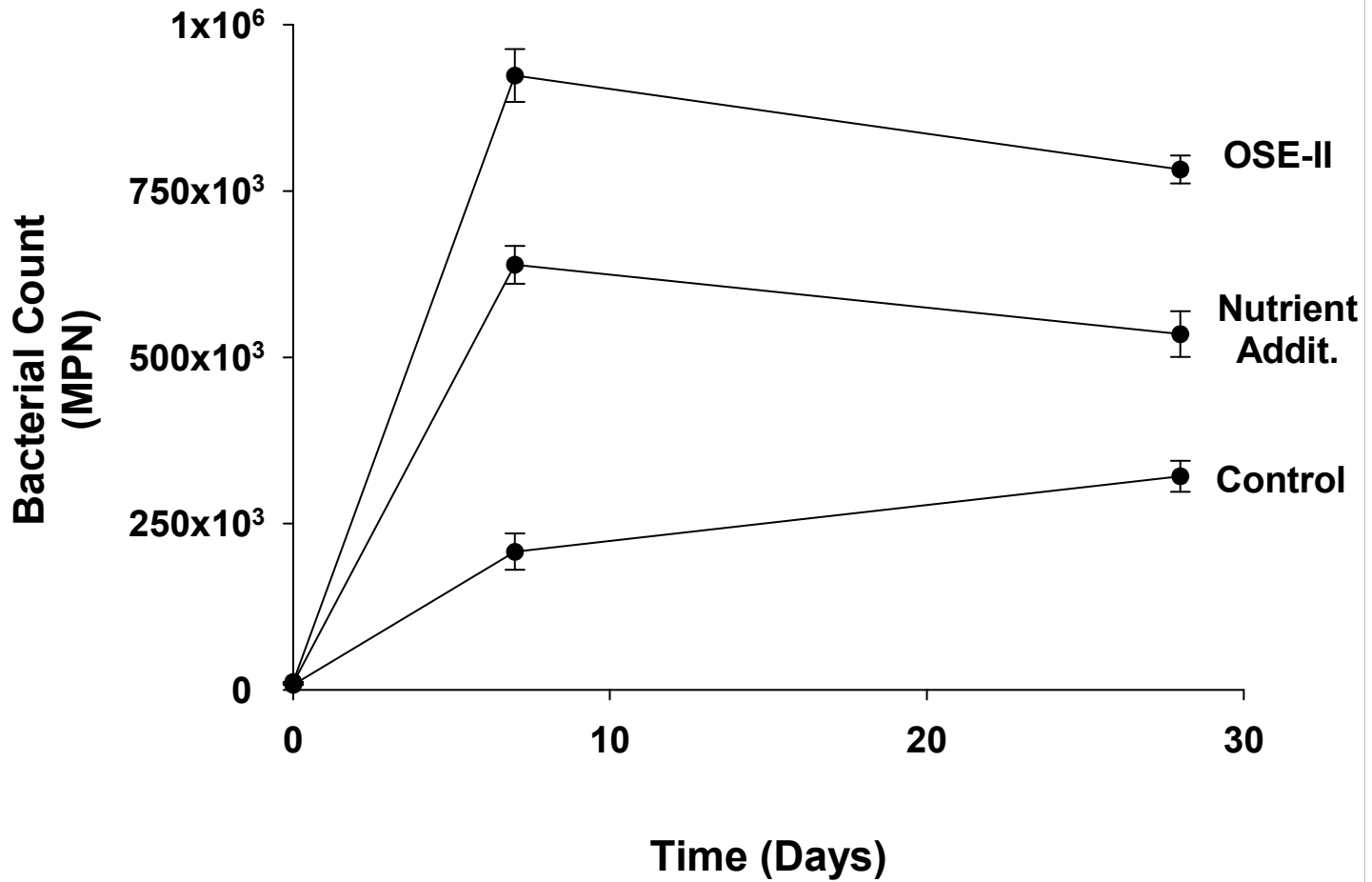
Bacterial Enhancement

Enzymatic Bioremediation (OSE-II) Bacterial Enhancement



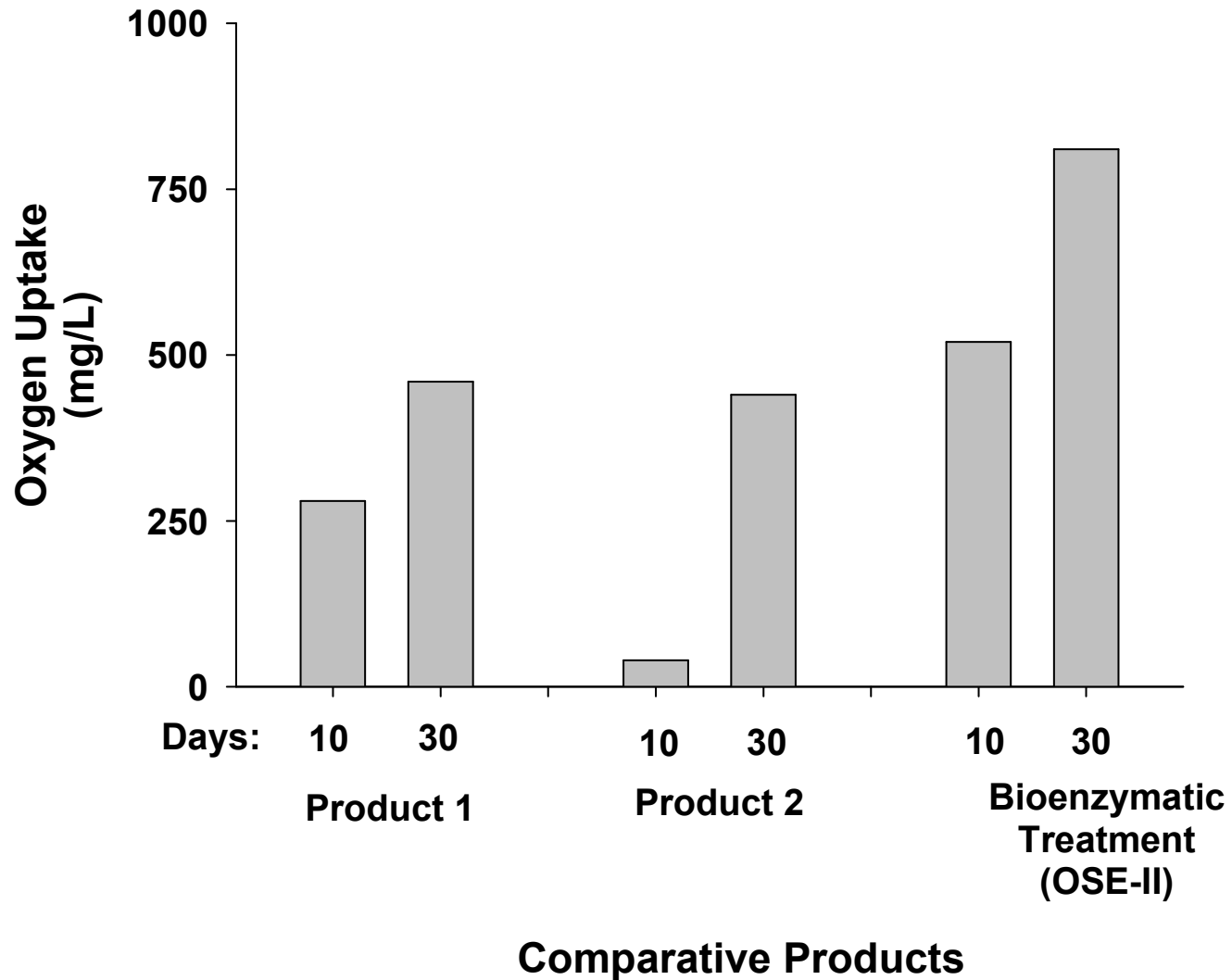
(Bio-Aquatic Testing,
NELAC Certified)

Enzymatic Biotreatment (OSE-II) Bacterial Response



(U.S. Dept. Interior, BOEM; and
Dept. Env'tl. 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 recovery, 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)**

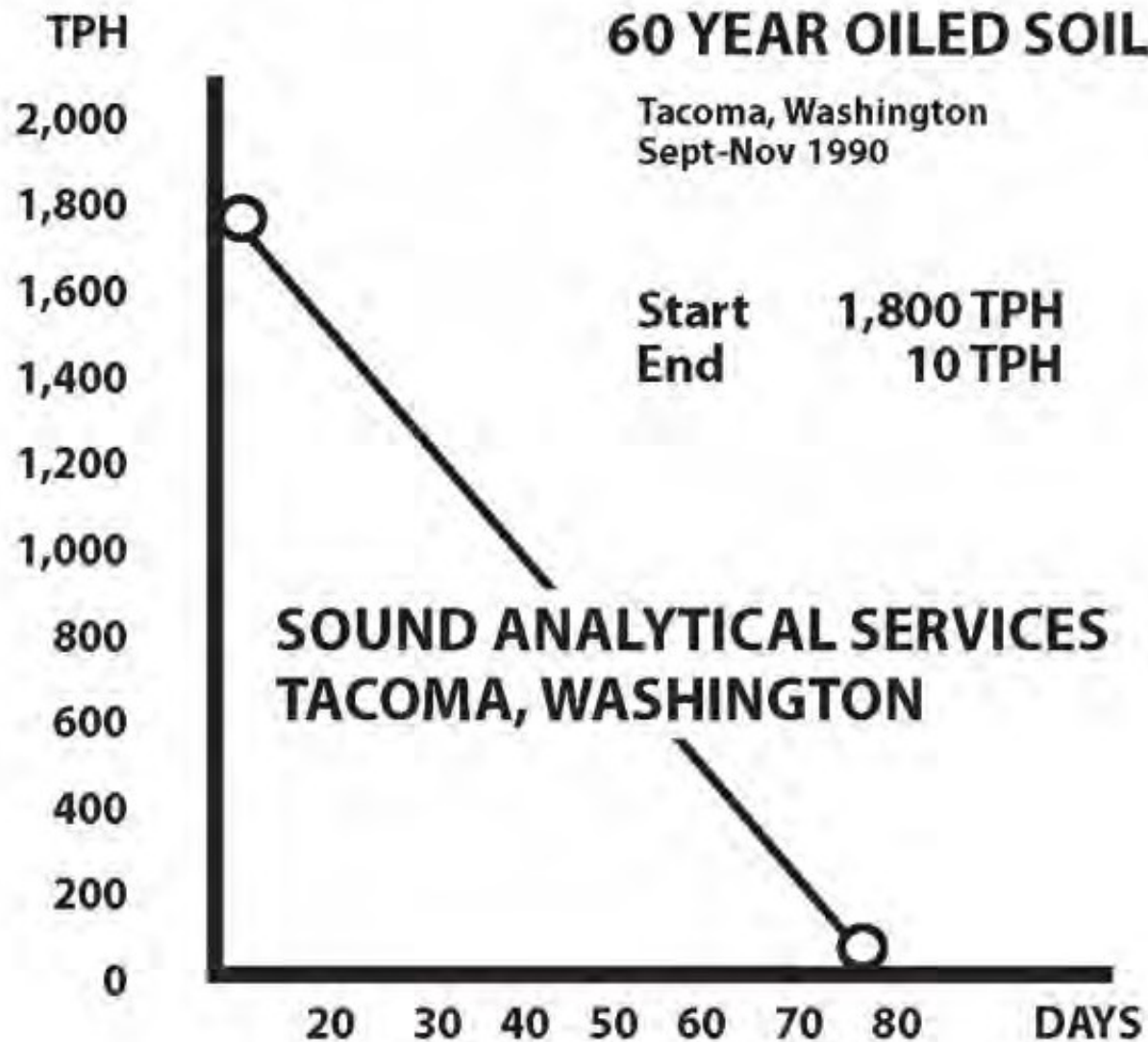




Acknowledgements

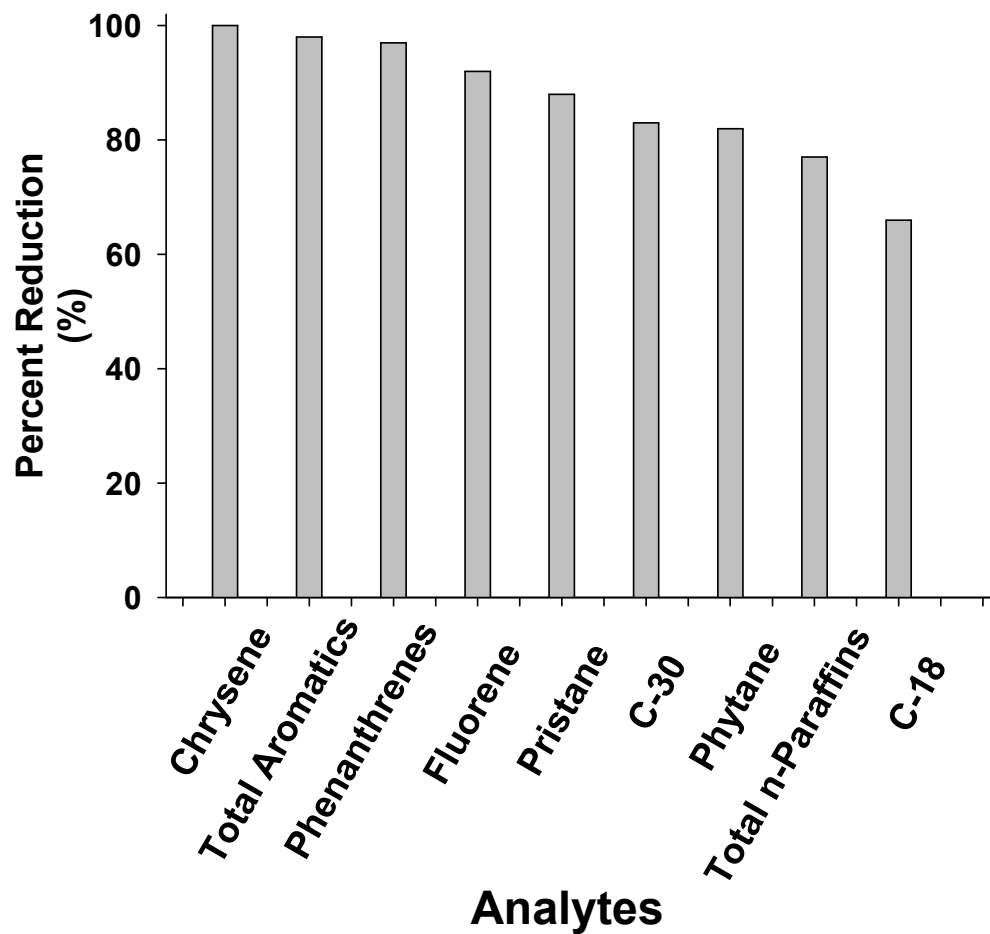
- The Lawrence Anthony Earth Organization (LAEO)
 - Ms. Diane Wagenbrenner
 - Ms. Elizabeth Montgomery
 - Ms. Barbara Wiseman
- Mr. Steven Pedigo, The OSE-II Corporation
- Louisiana Universities Marine Consortium (LUMCON)
- The 2016 Clean Gulf Conference – Ms. Cassie Davie

	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)



**Tier II Efficacy Data
Enzymatic Bioremediation Treatment
(OSE-II)**

Percent Reduction after 21 Days



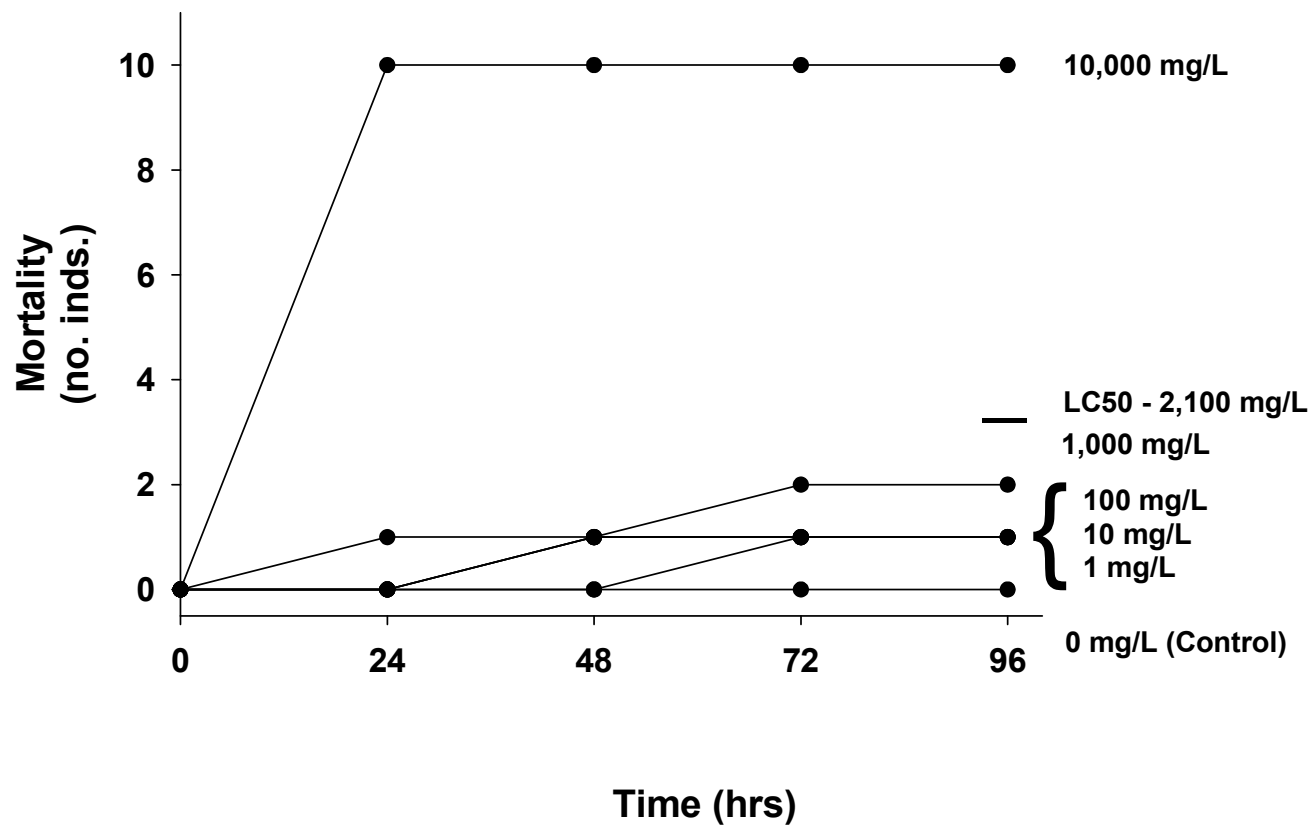
OSE-II Toxicit

Menidia ber

(Silve



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



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**