13127 Chandler Drive
Dallas, Texas 75243

(972) 669-3390
(972) 644-8359 (Fax)

OSEI

OIL SPILL EATER INTERNATIONAL, CORP.

OIL SPILL EATER II®

TECHNICAL INFORMATION

Natural Biological Enzyme

Revised April 2002

PATENT PENDING

OIL SPILL EATER INTERNATIONAL, CORP.

13127 Chandler Drive
Dallas, Texas 75243
(972) 669-3390
(972) 644-8359 Fax
admirallively@msn.com

COMPANY HISTORY

The ***Oil Spill International Corporation*** (originally Sky Blue Chemicals) began operation in 1989. The OSEI Corporation manufactures and markets a bioremediation product, ***Oil Spill Eater II***, which is an EPA listed liquid nutrient with enzymes for cleaning up hydrocarbons or other organic base contaminants.

OSE II is not a fertilizer or a bacterial product, but rather vigorously stimulates the growth of indigenous (local) bacteria, which bacteria, then devour the contaminant, with the only harmless residues left being carbon dioxide and water.

The OSEI Corporation is marketing OSE II throughout the world. All five branches of the United States Military use OSE II, and OSE II has also been used by the Canadian military.

Our overseas market is growing rapidly as the OSEI Corporation continues to prove to and demonstrate to new customers that ***Oil Spill Eater II*** is the most tested, most effective and efficient bioremediation product in the world.

We know you will agree that the information in this Technical Package contains the most thorough and extensive test data and application information published for any bioremediation product.

In this Package we show you why OSE II is so significantly better than bacterial products, fertilizers, nutrients, dispersants and surface washing agents. Even the U.S. Environmental Protection Agency proves to you that bacterial products and nutrients simply do not work.

We urge you to use ***Oil Spill Eater II*** for cleaning up hydrocarbon and virtually all organic based contaminants. We know you will find OSE II to be as effective as we claim.

Sincerely,

O.A. (George) Lively
Rear Admiral (RET)
President

OAL/eem

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April 25, 2002

USING CONTRACTORS

It has been our experience in the U.S. since 1989, that cleanup contractors do not want to use OSE II because it is too cost effective and efficient.

One large contractor, who tested OSE II, said *"It works great, but we make more money the old-fashioned way,"* i.e. using lots of labor, material, and time. Their preferred method is to dig and haul.

Unfortunately, when you *"dig and haul"* your contamination, all you have done is move your problem and you still own the contaminated soil!

Contractors should use OSE II. It would allow them to do a more effective cleanup and would allow them to earn more profit.

You should require that your cleanup contractors use OSE II! It will save you money and you eliminate your problem - **permanently**!

O.A. (George) Lively
Rear Admiral (RET)
President

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INTRODUCTION

This *Technical Package* contains a multitude of tests proving that ***"OIL SPILL EATER II"*** (OSE II) can rapidly and effectively mitigate hydrocarbons from Alaskan Crude Oil to BETX, as well as many other organic contaminants.

When comparing our product to competitors, we urge you to ensure you are comparing *"apples"* to *"apples."* Please consider that OSE II:

1. Is one of the ten biological products tested by the EPA at NETAC (National Environmental Technology Applications Center) listed at the University of Pittsburgh Applied Research Center. NETAC chose the ten best biological additives to test.

2. Has been tested by the EPA/NETAC as a biological agent and proved to biodegrade oil (hydrocarbons) and to be non-toxic. The attached EPA/NETAC Test Reports prove that OSE II does biodegrade oil (Alaskan Crude) and is non-toxic.

3. Does not just biodegrade "itself" and leave the hydrocarbon or contaminant. Many competitors are selling "soap" at $600.00 per drum. OSE II does (in fact) biodegrade the contaminant.

4. Uses "indigenous" bacteria to biodegrade the hydrocarbon. OSE II grows indigenous bacteria rather than introducing foreign "bugs" into the local ecosystem.

5. Is not listed as a dispersant by the EPA, since a dispersant will simply break up the contaminant (oil) and sink the oil, but will not stimulate biodegradation.

6. Is not a fertilizer since ***"OIL SPILL EATER II"*** gives you the following benefits over using fertilizers:

   A. COST CONTROL. We know how much "OSE II" is required on any given spill.

   B. OWN CARBON SOURCE. OSE II contains its own carbon which aids in bacterial growth.

C. BACTERIA. OSE II uses indigenous bacteria.

D. PRODUCT ADHERES TO OIL. OSE II molecularly adheres to hydrocarbons.

E. CATALYST. OSE II enzymes are catalysts for breaking down hydrocarbon walls and rapid bacterial growth.

F. REDUCES FIRE HAZARD 3 minutes after being applied.

G. REDUCES hydrocarbon's adhesion properties.

7. Is not a bacterial product since:

A. Fresh diesel or other hydrocarbons will kill bacterial products.

B. Bacterial products cannot determine how much product to use.

C. Foreign bacteria and indigenous bacteria will fight each other for the food source, and the U.S. EPA claims the indigenous bacteria will overtake the added non indigenous bacteria.

8. Last, but very important, how much product do you need (cost) to biodegrade a gallon or cubic yard of contaminant? With OSE II you know exactly because the information is published in our literature.

We hope this will assist you in your decision process in purchasing a bioremediation product that is time, cost and environmentally effective. We urge you to go with a proven product ***"OIL SPILL EATER II"***.

Thank you for your consideration.

Sincerely,

O.A. (George) Lively
Rear Admiral (RET)
President

OAL/eem

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OIL SPILL EATER II
May - 1993
GENERAL DESCRIPTION

OIL SPILL EATER II is a unique Biocatalytic System of preformed multi-enzyme liquid concentrate. OIL SPILL EATER stimulates and accelerates natural biological reactions. When combined with fresh or salt water and oxygen, OSE II will cause crude oil and other organic substances to rapidly decompose; eventually biodegrading them to carbon dioxide and water.

OIL SPILL EATER II is non-toxic to humans, animals, plants and marine life. It is non-poisonous, even if accidentally ingested. It is non-irritating to the most sensitive skin. OSE II contains no known allergens to cause skin, respiratory or other allergic reactions. Birds bathed in OSE II should be quarantined until their own natural oils are restored.

OIL SPILL EATER II is 100% Biodegradable. OSE II has a 5 year shelf life when stored at temperatures below 120 degrees F. Freezing does not harm OSE II; however, cold temperatures slow it's reaction rate somewhat. The product is completely stable and reactive in a pH environment of 3.5 to 11.7.

OSE II contains no corrosive chemicals or metal trace elements, and will not damage electrical insulation or painted surfaces. No special protective clothing or safety equipment is required - as determined by OSHA - Anchorage, Alaska.

OSE II assists in controlling unpleasant odors associated with hydrocarbons. OSE II will destroy - not mask - odors through a natural Biodegradation process.

OSE II will reduce fire hazard once emulsification and the solubilization process is started. This process begins the instant OSE II is applied to crude oil, gasoline or spilled hydrocarbons.

OSE II can be applied easily using a pumper-truck or fire hose, or even a pump-up hand sprayer. On water, OSE II can be applied by omni barge, helicopter, plane or any eductor system.

OSE II eliminates the need for skimmers and it eliminates the problem of disposal (clean docks, driftwood, boats, rubber gear and shorelines). No secondary cleanup is required because OSE II converts the hydrocarbons to C02 and water.

OIL SPILL EATER II
May - 1993
GENERAL DESCRIPTION
(Continued)

AGE OF CONTMINATED HYDROCARBONS

The older or more weathered hydrocarbon contamination increases the time for Bioremediation to occur. When contamination is exposed to the open air and weather, it can form a skin, similar to the way gelatin sets up. The older the hydrocarbon and the more it is exposed to the elements, the thicker the skin becomes; hence, eventually becoming asphaltenes. The thicker this skin - the longer Bioremediation will take to reduce the contamination's TPH. Therefore, the sooner a contamination is addressed and mitigated, the contamination cleanup will be less expensive and less time consuming.

Steven R. Pedigo
Chairman
OSEI, Corp.

SRP/AJL

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CHEMICAL PROCESS

February 1991

Once OIL SPILL EATER II is applied to a hydrocarbon spill, the enzymes and other product constituents start emulsification and solubilization of the hydrocarbon substrate. Emulsification and solubilization generally take from a few minutes up to a few hours for weathered heavy-end hydrocarbons, once OSE II is applied with a temperature of 40 degrees F. or greater. Once solubilization is completed, the hydrocarbon substrate is less toxic (and the hazard of fire is diminished) so the enhanced - naturally occurring bacteria will have a higher affinity for the solubilized hydrocarbon substrate.

NOTE: There is no hydraulic loading with the use of OSE II and therefore related hydrocarbons are not pushed into the lower depths of the water column. During these reactions, OSE II offers up a complete nutrient system to promote the rapid growth or colonization of naturally occurring indigenous bacteria.

OSE II is also formulated so that once application to the hydrocarbon substrate occurs, molecular adhesion takes place. This prevents OSE II from being removed from the hydrocarbons easily. The above reaction forms the substrate complex.

Once the outer molecular walls of the hydrocarbon substrate complex have been weakened or broken, then this allows bacteria better access to the hydrocarbon substrate. The nutrients in OSE II's product matricies (readily available nitrogen, phosphorous, carbon and vitamins) rapidly populates naturally occurring bacteria. There are certain product constituents to specifically enhance various hydrocarbon degrading bacteria. The naturally enhanced hydrocarbon degrading bacteria rapidly populate until product nutrients are depleted, at which time they readily convert to the only food source left (the weakened or broken hydrocarbon substrate). The transition- state complex is when the enhanced naturally occurring hydrocarbon degrading bacteria start converting hydrocarbons to C02 and water.

Chemical Process (continued)
February 1991

The enhanced naturally occurring hydrocarbon degrading bacteria convert the solubilized hydrocarbons to C02 and water which is the end point or the Bioremediation of the hydrocarbon substrate. Any OSE II product components left are 100% Biodegradable and will be used up naturally.

This process emulates Mother Nature completely!

NOTES:

OSE II's optimum temperature range is 40 degrees F. to 110 degrees F. - however OSE II is effective in the range of 28 degrees F. to 120 degrees F.

OSE II has a five (5) year shelf life if stored in a covered area where the temperature does not exceed 102 degrees F.

Our research has determined that the age and weathering of hydrocarbons (if weathered over 1 to 2 years) may slow Bioremediation somewhat.

By: Steven R. Pedigo
Chairman
OSEI, Corp.

SRP/AJL

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EMULATING MOTHER NATURE

HOW BIOREMEDIATION OCCURS IN MOTHER NATURE

We would like to first explain what happens in Mother Nature when a hazardous material is spilled.

There is a myriad of bacteria everywhere, where the spill comes in direct contact with bacteria; that bacteria is killed or dies off. The bacteria that is proximal to the spill but not in direct contact reacts in several ways.

First, the bacteria separate themselves far enough away so as to protect themselves from the toxicity of the spill.

Second, the bacteria then release enzymes and bio surfactants to attack the spill.

Third, the bio surfactants emulsify and solubilize the spill. What this means is the bio surfactants will break up the spill and partition the spill into a manageable consistency. This is also breaking down the molecular structure of the spill or detoxifying it, so it can be used as a food source.

The enzymes then form binding sites on the emulsified or solubilized spill and this is where the bacteria will initially attach themselves and start the digestive process.

For this process to occur there has to be large amounts of bacteria and it is a long process for bacteria to acclimate themselves to a spill. Then it takes time for the bacteria to release enzymes and surfactants. One of the limiting factors is the number of bacteria present to produce and release enough enzymes and surfactants to get the process started. This is why you hear scientists talk about adding nutrients to jumpstart the rapid growth of bacteria so enough enzymes and bio surfactants can be released to affect the mitigation of the spill.

However, nutrients alone are limited because of concentration (washed away or diluted) and the time it takes to grow a large population of bacteria.

Wouldn't it be nice if there was a means of emulating Mother Nature and at the same time speeding up the process to mitigate in hours or days what Mother Nature takes days, months and years to handle on her own?

OIL SPILL EATER II

We have a product that contains the enzymes, bio surfactants, nutrients and other necessary constituents for *complete life cycles and biodegradation*. When our product is added to a spill, it is not necessary to wait on the proximal bacteria to release enough enzymes or bio surfactants since they are already supplied in our product. Therefore, the minute you apply OSE II, there is enough bio surfactant to start the emulsification and solubilization process. This process generally takes a few minutes to several minutes, depending on the consistency of the spill. As the bio surfactants do their job, the enzymes are attaching themselves to broken hydrocarbons, forming digestive binding sites.

Note: *Once this process has occurred, several things are true:*

1. The fire hazard has diminished.
2. The toxicity of the spill is rapidly diminished.
3. The odor or smell is almost non-existent.
4. The oil or spill will no longer adhere to anything.

If the spill has not reached a shoreline yet, but does so after application, it will not adhere to sand, rock, wood, metal or any vegetation.

If the spill has already attached itself once application occurs, the spill will be lifted from sand, rock, wood, metal or vegetation.

The spill is detoxified to the point, that indigenous (natural) bacteria can now utilize the oil as a food source. Also, this diminishes toxicity to marine organisms, birds or wildlife.

OSE II causes the oil to float on the surface of the water, which reduces the impact to the sub-surface - preventing secondary contamination of the water column or tiertiary contamination of the floor of the body of water at the spill area.

The spill being held on the surface will make it easy to monitor. You will be able to see bacteria growing on the spill, and the oil will be digested to CO_2 and water before your eyes on a contained spill.

Unlike mechanical cleanup, which cleans up a maximum of 20% of the oil spilled, OSE II will clean up 100% of the spilled oil.

Oil Spill Eater II emulates (copies) mother nature's process exactly.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

AUG 26 1996

OFFICE OF
SOLID WASTE AND EMERGENCY
RESPONSE

Mr. O.A. Lively
OSEI Corp.
5545 Harvest Hill
Suite 1116
Dallas, TX 75230

Dear Mr. Lively:

Thank you for providing the technical product data required by the revised National Oil and Hazardous Substances Pollution Contingency Plan (NCP), 40 CFR Part 300, on the Bioremediation Agent "Oil Spill Eater II (OSE II)." Your data submission satisfies the requirements contained in Title 40 of the CFR section 300.915 of the NCP. "Oil Spill Eater II (OSE II)" will be listed on the NCP Product Schedule under Bioremediation Agents and may be authorized for use by Federal On-Scene Coordinators in accordance with 40 CFR section 300.910. The technical data for this product will be kept on file by the Oil Program Center pursuant to 40 CFR section 300.920.

Enclosed are some of the relevant provisions in the NCP on restrictions regarding the listing of your product. Please note, you are required to notify the Environmental Protection Agency (EPA) of any changes in composition, formulation, handling procedures, or application of your product. Based on this notice, EPA may require retesting of the product. Also, note that the listing of "Oil Spill Eater II (OSE II)" on the NCP Product Schedule does not constitute approval, certification, authorization, licensing or promotion of the product; nor does it imply compliance with any criteria or minimum standards for such agents. Failure to comply with these restrictions or the making of any improper reference to EPA in an attempt to demonstrate approval or acceptance of the product will constitute grounds for removal of the product from the schedule.

If you have questions, please contact Ms. Gail Thomas in the Oil Program Center at (703) 603-8790 or our information line at (202) 260-2342.

Sincerely,

David Lopez, Director
Oil Spill Prevention and Response Center (5203G)

Enclosure

OIL SPILL EATER INTERNATIONAL, CORP.

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admirallively@msn.com

January 16, 2000

EPA REGULATIONS

The statement (direct quote) below was received from Mr. Donald Smith of REGION VI of EPA's Superfund Division, Response and Prevention Branch on January 11, 2001.

This statement is quite clear that for oil spills on soil, concrete or asphalt (or other surfaces that do not involve U. S. Navigable Water) the Spiller is free to use OIL SPILL EATER II to clean up the spill.

O. A. (George) Lively

"The United States Environmental Protection Agency's policies for the use of dispersants, bioremediation and chemical agents for responding to spills of petroleum or vegetable oils are outlined in Title 40 Code of Federal Regulations Part 300.900 which is entitled the National Contingency Plan. The use of these products relative to federal authorities is regulated to spills that threaten to or have spilled into or upon the waters of the United States. The definition of the waters of the United States can be found in Title 40 Code of Federal Regulations Part 110.2 . Generally speaking, the waters of the US include, but are not limited to lakes, rivers, streams, creeks, city storm sewers and drainage ditches. If a discharge threatens or has discharged as described in the aforementioned scenario, then the use of chemical or biological agents must have the permission of a Federal On Scene Coordinator. If the discharge does not meet these parameters as previously described then the permission of the Federal On Scene Coordinator is not required. However, please be advised that there are state and local authorities/ordinances that may require a permit or some of pre application approval notice."

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April 26, 2002

To: ALL USERS OF "*OIL SPILL EATER II*" (OSE II)

All storm drains discharge into the navigable waters of the United States.

The OSEI Corporation guarantees that OSE II, after being properly applied, will biodegrade all non-halogenated hydrocarbons (such as gasoline, jet fuels, diesel, ethylene glycol, crude oil, hydraulic fluid, engine oil, etc.) and some halogenated hydrocarbons. OSEI Corporation further guarantees that these treated contaminants, when washed down storm drains, will have no adverse effect on the environment, nor endanger life, health, or property or constitute a public nuisance, and will have toxicity levels substantially below the toxicity level already established by the Environmental Protection Agency (EPA) as the acceptable standard for the navigable waters of the United States.

That standard is currently 2.61 as determined by the EPA's LC50 Test on the following page. "*OIL SPILL EATER II's*" toxicity value, using the same LC50 Test on the same species, is 2900 (the lower the LC50 test value, the higher the toxicity).

For specific application details, please contact our office.

Sincerely,

Steven R. Pedigo
Chairman

SRP/eem

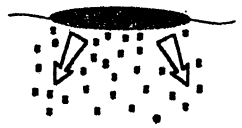

Table 14. Characteristics of Dispersants Listed on the NCP Product Schedule (as of August 1999).

	Corexit 9500	Corexit 9527	Dispersit SPC	Mare Clean 200	Neos AB 3000
Dispersant Type	Concentrate; solvent is ethylene glycol monobutyl ether	Concentrate; solvent is ethylene glycol monobutyl ether	(Just added in April 1999) Concentrate; solvent is water based	Concentrate; solvents are paraffinic hydrocarbons	Concentrate; solvents are paraffinic hydrocarbons
Availability	Get from NSFCC	Get from NSFCC	NP	Get from NSFCC	Get from NSFCC
Application Rate	Apply undiluted at 2-10 gal per acre, or a dispersant:oil ratio of 1:50 to 1:10	Apply undiluted at 2-10 gal per acre, or a dispersant:oil ratio of 1:50 to 1:10	Apply at 2-10 gal per acre; or dispersant:oil ratio of 1:50 to 1:10	Apply a dispersant:oil ratio of 1:5 (53-66 gal per ton of oil)	Apply a dispersant:oil ratio of 1:4 to 1:2.4 (75-125 gal per ton of oil)
Application Method	Spray neat as droplets	Spray neat as droplets	Spray neat as droplets	Spray neat as droplets	Spray neat as droplets
Temperature Limitations	Above -30°F	Above -30°F	Above - 25°F	Above 21°F	Above 32°F
EPA Dispersant Effectiveness Test (%)	Prudhoe Bay crude: 45 S. Louisiana crude: 55 Average of above: 50	Prudhoe Bay crude: 37 S. Louisiana crude: 63 Average of above: 50	Prudhoe Bay crude: 52 S. Louisiana crude: 50 Average of above: 51	Prudhoe Bay crude: 64 S. Louisiana crude: 84 Average of above: 74	Prudhoe Bay crude: 20 S. Louisiana crude: 90 Average of above: 55
Vendor Lab Report on Effectiveness (%)	Prudhoe Bay crude: 45 S. Louisiana crude: 55 Average of above: 50	Prudhoe Bay crude: 37 S. Louisiana crude: 63 Average of above: 50	Prudhoe Bay crude: 40 S. Louisiana crude: 105 Average of above: 73	NP	NP
Use in Fresh Water?	Not effective	Not effective	NP	Not effective	Not effective
Use in Salt Water?	Yes	Yes	YES	Yes	Yes
Worker Safety (Level of Protection)	Level D	Level D	Level D	NP	NP
NCP Reported Toxicity of Dispersant Alone (LC-50, ppm) Note: a low value = high toxicity					
Inland silversides (96h)	25.2	14.6	3.5	1,996	91.1
Mysid shrimp (48h)	32.2	24.1	16.6	938	33
NCP Reported Toxicity of Dispersant & No. 2 Fuel Oil (1:10 ratio) (LC-50, ppm) Note: a low value = high toxicity					
Inland silversides (96h)	2.61	4.49	7.9	42.0	57.0
Mysid shrimp (48h)	3.4	6.6	8.2	9.84	25.0

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard, 29 CFR 1910.1200. Standard must be consulted for specific requirements.

U.S. Department of Labor
Occupational Safety and Health Administration
(Non-Mandatory Form)
Form Approved
OMB No. 1218-0072

IDENTITY *(As Used on Label and List)*

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I OIL SPILL EATER II

Manufacturer's Name OIL SPILL EATER INTERNATIONAL	Emergency Telephone Number (972) 669-3390
Address *(Number, Street, City, State, and ZIP Code)* 13127 Chandler Drive	Telephone Number for Information same - FAX (972) 644-8359
Dallas, Texas 75243	Date Prepared October 28, 1998
	Signature of Preparer *(optional)* Steve Ledyard

Section II — Hazardous Ingredients/Identity Information

Hazardous Components (Specific Chemical Identity; Common Name(s))	OSHA PEL	ACGIH TLV	Other Limits Recommended	% *(optional)*
No Hazardous Components (OSE II)	NO TLV	NO TLV	NONE	
H2O	NO TLV	NO TLV	NONE	
NITROGEN	NO TLV	NO TLV	NONE	
MOLASSES	NO TLV	NO TLV	NONE	
NON IONIC SURFACTANT	NO TLV	NO TLV	NONE	
SUGAR	NO TLV	10 mg. per Cubic M. dry	NONE	
PROTEASE	NO TLV	NO TLV	NONE	
PHOSPHORUS	NO TLV	NO TLV	NONE	
YEAST	NO TLV	NO TLV	NONE	
AMYLASE	NO TLV	NO TLV	NONE	
ANIONIC SURFACTANT	NO TLV	NO TLV	NONE	
MALT	NO TLV	NO TLV	NONE	

Section III — Physical/Chemical Characteristics

Boiling Point	214°F.*	Specific Gravity (H_2O = 1) 20° C	1.05
Vapor Pressure (mm Hg.)		Melting Point	.0°F.
Vapor Density (AIR = 1)	1.1	Evaporation Rate (Butyl Acetate = 1)	

Solubility in Water: 100%

Appearance and Odor: Amber with the small of some ferment.

Section IV — Fire and Explosion Hazard Data

Flash Point: (Method Used)*	Flammable Limits	LEL	UEL
In excess - 7000°F - fire retardant	NON FLAMMABLE	NA	NA

Extinguishing Media: NONE - FIRE RETARDANT *METHOD-ASTM-D56

Special Fire Fighting Procedures: NONE - FIRE RETARDANT

Unusual Fire and Explosion Hazards: NONE

(Reproduce locally)

OSHA 174, Sept. 1985

Section V — Reactivity Data

Stability	Unstable		Conditions to Avoid: Temperature above 120°F can reduce
	Stable	X	enzyme activity, avoid acidic conditions below 3.5pH

Incompatibility (*Materials to Avoid*): strong bases over 11.7.
strong bases over 11.7.

Hazardous Decomposition or Byproducts: NONE (By-products CO2 and water).

Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	

Section VI — Health Hazard Data

Route(s) of Entry — Inhalation? Non-toxic — Skin? non-toxic — Ingestion? Toxic if more than one (1) quart injested.

Health Hazards (*Acute and Chronic*): Toxicity tests - Inhalation, skin sensatization, occular, and ingestion show virtually no toxicity.

Carcinogenicity: None — NTP? No listing. — IARC Monographs? None — OSHA Regulated? No

Signs and Symptoms of Exposure: N/A

Medical Conditions Generally Aggravated by Exposure: NONE

Emergency and First Aid Procedures: Wash eyes thoroughly. Use good hygenic practices.

Section VII — Precautions for Safe Handling and Use

Steps to Be Taken in Case Material Is Released or Spilled: Can be washed into sewer systems, or absorbed by earth.

Waste Disposal Method: No special disposal.

Precautions to Be Taken in Handling and Storing: Handling - none. Do not store where temp. exceeds 120°F./5 Year Shelf Life

Other Precautions: NONE

Section VIII — Control Measures

Respiratory Protection (*Specify Type*): None required.

Ventilation	Local Exhaust: Not required	Special: None
	Mechanical (General): Not required.	Other: None

Protective Gloves: Not required. — Eye Protection: Not required.

Other Protective Clothing or Equipment: None

Work/Hygienic Practices: Use good normal hygenic practices.

STEVE COWPER, GOVERNOR

DEPARTMENT OF LABOR

OCCUPATIONAL SAFETY AND HEALTH
LABOR STANDARDS AND SAFETY DIVISION

3301 EAGLE STREET, SUITE 303
PO. 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

OIL SPILL EATER INTERNATIONAL, CORP.

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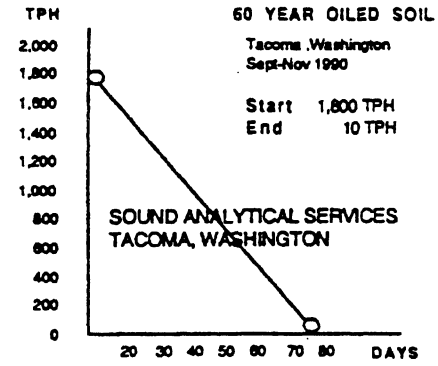
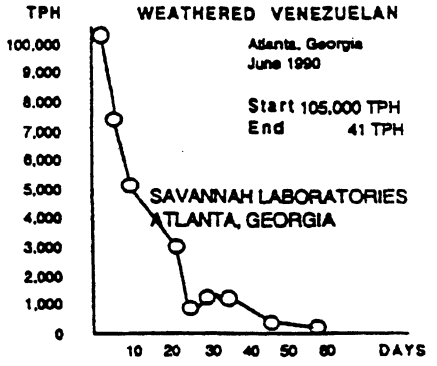
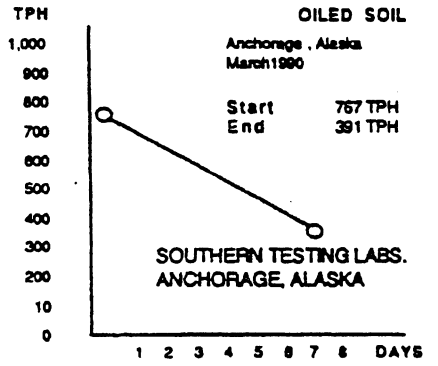
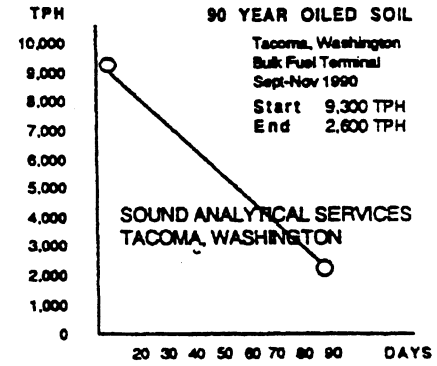
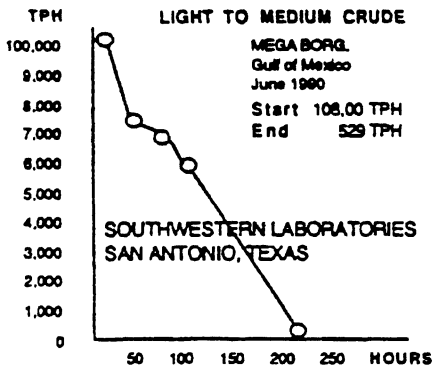
TPH BIODEGRADATION TESTS OF OSE II PERFORMED BY INDEPENDENT LABORATORIES

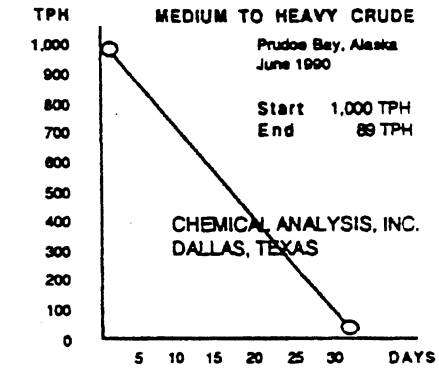












This page represents a sampling of laboratory test reports available upon request.

OIL SPILL EATER INTERNATIONAL, CORP.

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(972) 644-8359 Fax
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SUMMARY

U.S. EPA and NETAC EFFICACY TESTING

The United States Environmental Protection Agency spent one and one-half years testing and evaluating protocols using OIL SPILL EATER II.

Mr. Tom Merski (August 18, 1993) explained the control (oil and seawater only) showed such an insignificant change (no reduction in TPH) that the control results were not even released.

NOTE - that OIL SPILL EATER II Biodegraded Alaskan Crude Oil 98% in 21 days in NETAC's Tier II Test. This test specifically shows the reduction of Polynuclear Aromatic Hydrocarbons that are the Hydrocarbons that are more persistent and difficult to Bioremediate!

This test proves that using OIL SPILL EATER II is beneficial over doing nothing, and that 98% of a spill can be mitigated as opposed to mechanical cleanups, which after 30 days or more can only blot up 20% or a spill. Using OIL SPILL EATER II can reduce the impact to marine organisms and ECO Systems faster and more efficiently than mechanical cleanups. This means huge savings on the cleanup costs and environmental damage assessment fees.

By: Steven R. Pedigo
Chairman
OSEI, CORP.

SRP/AJL

NETAC

National Environmental Technology Applications Center
UNIVERSITY OF PITTSBURGH APPLIED RESEARCH CENTER
615 William Pitt Way • Pittsburgh, PA 15238
Facsimile (412) 826-5552
(412) 826-5511

July 22, 1993

Mr. George Lively
President
OSEI Corporation
Oil Spill Eater International
Suite 1116, 5545 Harvest Hill
Dallas, TX 75230

New address as of Oct. 1999
13127 Chandler Drive
Dallas, TX 75243

Dear Mr. Lively:

Subject: ***Oil Spill Eater II Methods Validation Data***

Per your request, enclosed is the efficacy data generated with "Oil Spill Eater II" from the development and validation of our oil spill response bioremediation evaluation methods. The toxicity data from this process will be provided as soon as it is released from the EPA Office of Research and Development laboratories. We have included information on the experimental methods and objectives intended to assist you in understanding the meaning of the numbers generated for this report.

On behalf of NETAC and all the members of our Oil Spill Product Protocol Development Panel, we wish to express our appreciation for the contribution of your bioremediation agent for use in validating these methods and for your availability to answer questions about how this agent was intended to be used. Your patience and cooperation over the past two years has been commendable.

As you are aware, these experiments were conducted by the NETAC and EPA Office of Research and Development laboratories in Cincinnati, OH and in Gulf Breeze, FL. These data give you a general idea of how your product may behave in an open environment. Note that these data were obtained during the development of our methods. Numerous refinements have been made to increase the sensitivity of these tests; therefore, your product may provide different results in future tests due to this increased sensitivity as well as from the natural variability of the product and the constituent(s) used in the test sequence.

Please bear in mind that, although the Tier II methods have been finalized, we anticipate that all of the methods will be refined and updated periodically as we learn more about these systems. This means that data which was incidentally obtained for your product during the development of the protocols as it currently stands may change as the protocol is further refined. We must emphasize the research nature of the data we are providing to you today!

These data are provided to give you an indication of how your product behaved in this particular phase of the research. Different results may occur with the newly refined methods. We recommend that you evaluate this information as another set of intermediate data. We strongly suggest that you initiate additional testing applying the final Tier II method to develop a product performance baseline.

We also wish to emphasize that the participation of any bioremediation agent in the development or validation of the protocol does not constitute endorsement, approval or recommendation on the part of either NETAC or the EPA Office of Research and Development.

Enclosed for your convenience are the tabulated results of the Day 21 shaker flask experiment for efficacy testing, and a Statistical Method Summary used to generate data about your product. This statistical method can be found in the July 1993 issue of the *Evaluation Methods Manual for Oil Spill Response Bioremediation Agents*. This document is currently being printed and a copy of the manual will be sent to you as soon as possible.

If you have any questions about the data which we have provided, its potential use or application, or development of the protocol please call me at (412) 826-5511.

Sincerely,

A. Thomas Merski
Vice-Chairman,
Treatability Protocol Development Subcommittee,
Bioremediation Action Committee

ATM\MRM:tmw
H:\public\bpec\OSEI-2.ltr
310-2015-141

cc: W.M. Griffin

RESULTS:

TIER II EFFICACY DATA

PERCENT REDUCTION

OIL SPILL EATER II (Day 21)

ANALYTE	LAB A (n = 3) (%)
PRISTANE	88
C18	66
PHYTANE	82
C30	83
TOTAL n-PARAFFINS	77
FLUORENE	92
PHENANTHRENE	97
CHRYSENE	165
TOTAL AROMATICS	98

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SUMMARY

SECOND U.S. EPA/NETAC (Bioremediation Test)
Using OIL SPILL EATER II
February 28, 2001

The second U.S. EPA/NETAC Test was more thorough with different days for testing the amount of bioremediation occurring. EPA/NETAC wanted to determine if there was a statistical difference between the control (doing nothing at all), the nutrient control (EPA - Dr. Venosa's nutrients) and the test product, **OIL SPILL EATER II**.

Table 2 shows the raw data where on day 0 the control, nutrient control and OSE II started at approximately 8,000 ppm (parts per million). In seven (7) days, OSE II had remediated the oil to an average of 6,529 ppm. The control and nutrient control were still around 8,000 ppm. On day twenty eight (28), OSE II had remediated the oil to 3,658 ppm. While the control was where it started and the nutrient control showed only minimal reduction of the oil.

In fact, OSE II remediated more of the oil in seven (7) days than the nutrient or nutrient control remediated in twenty eight (28) days.

EPA/NETAC through scientifically valid testing wanted to determine through an Anova Table if there was significant statistical difference between the nutrient, nutrient control, and the test product, OSE II.

In this very limited closed system, OSE II reduced the oil over 50%, while very little reduction occurred in the control or nutrient control. In fact, on Page 3, in the last paragraph, EPA/NETAC explains that for OSE II (Group 3) "at day 7 and day 28 are significantly different from (Group 1) and (Group 2)."

This test is reproduced as the example in the U.S. Code of Federal Regulations under Bioremediation Efficacy Test.

EPA/NETAC conclude, "Therefore in terms of total aromatic degradation, the test indicates the desired statistically significant difference between the mean of the product (OSE II) and the mean of the non-nutrient control.

EPA/NETAC's scientifically valid Bioremediation Test proves that OSE II is a very significant oil spill cleanup product.

By: Steven R. Pedigo
Chairman

SRP/AJL

NETAC

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OIL SPILL RESPONSE BIOREMEDIATION AGENTS EVALUATION METHODS VALIDATION TESTING DISCUSSION OF RESULTS

The following data are provided for the oil spill response bioremediation agent producer as a means to begin to assess how this bioremediation agent may behave in response to an oil spill in the environment. The data we are providing are limited to the gas chromatographic/mass spectrometer (GC/MS) results.

Note that a total of 69 analytes (components naturally occurring in oil) were measured in these experiments. These analytes constitute a small but highly representative fraction of the toxic and biodegradable portion of oil. We are providing you with a summary of the ultimate results and a summary of the most germane analytes to facilitate our reporting of this information and to reduce confusion in reporting caused by the modification of the selected test dates.

The following table of GC/MS results indicate the percent reduction of analyte(s) versus the same analyte(s) present in the control (i.e., product results/control results x 100). For example, if 100 percent of an analyte is present at Day 21 after mixing oil, seawater and product as compared to the control (oil and seawater only) then the product did not stimulate the decomposition of hydrocarbons in oil. Note, that the greater the number of analytes with a low percentage the more capable the product of enhancing the biodegradation of oil.

From this experiment, the results indicated that there was sufficient comparability of the data between the laboratories conducting this experiment. The resultant data presented for this bioremediation agent and the comparative nutrient treatment did not show a significant statistical difference between the product mean and the control mean at the $p \leq 0.05$ level of significance. That is, biodegradation was occurring but not significantly faster than the control. We note that even though these treatments did not produce statistical significant degradation of the test oil, several of the products in this research did achieve this standard.

An analysis of the total aromatic data (in ppm) was conducted for the following three groups:

GROUP 1: Non-nutrient Control
GROUP 2: Nutrient Control
GROUP 3: Test Product — OSF II

The raw data is shown in Table 2 below. Note the three replications for each group-time combination.

TABLE 2

PRODUCT TEST DATA
TOTAL AROMATICS (PPM)

	GROUP 1	GROUP 2	GROUP 3
DAY 0	8153	7912	7711
	8299	8309	8311
	8088	8111	8200
DAY 7	8100	7950	6900
	8078	8200	6702
	7999	8019	5987
DAY 28	8259	8102	4000
	8111	7754	3875
	8344	7659	3100

Table 3 gives the summary statistics (number of observations, means, and standard deviations) for each group-time combination.

TABLE 3

SUMMARY STATISTICS FOR PRODUCT TEST DATA
TOTAL AROMATICS (PPM)

Time	Product	n	Mean	Standard Deviation
Day 0	Group 1	3	8180.0	108.1
	Group 2	3	8110.7	198.5
	Group 3	3	8074.0	319.2
Day 7	Group 1	3	8059.0	53.1
	Group 2	3	8056.3	129.1
	Group 3	3	6529.7	480.3
Day 28	Group 1	3	8238.0	117.9
	Group 2	3	7838.3	233.2
	Group 3	3	3658.3	487.6

Control — 0%

Nutrient — 3% ↓

OSF II — 54% ↓

NFTAC

Table 4 shows the results of the two-way ANOVA

TABLE 4

TWO-WAY ANOVA TABLE

Source	df	Sum of Squares	Mean Square	F-Statistic	p-value
GROUP	2	23944857.41	11972428.70	151.94	0.0001
TIME	2	10954731.19	5477365.59	69.51	0.0001
INTERACTION	4	19347589.04	4836897.26	61.39	0.0001
ERROR	18	1418303.33	78794.63		
TOTAL	26	55665480.96			

From the ANOVA table, we see that the F-statistic for INTERACTION is significant ($F = 61.39$, $p = 0.0001$). This indicates that group differences exist for one or more days. Protected LSD mean separations were then conducted for each day to determine which group differences exist. The results are summarized in Table 5. Note that means with the same letter (T grouping) are not significantly different.

TABLE 5

PAIRWISE PROTECTED LSD MEAN SEPARATION

T Grouping	Mean	n	Interaction
A	8238.0	3	Group 1, Day 28
A	8180.0	3	Group 1, Day 0
A	8110.7	3	Group 2, Day 0
A	8074.0	3	Group 3, Day 0
A	8059.0	3	Group 1, Day 7
A	8056.3	3	Group 2, Day 7
A	7838.3	3	Group 2, Day 28
B	6529.7	3	Group 3, Day 7
C	3658.3	3	Group 3, Day 28

Significance Level = 0.05
Degrees of Freedom = 18
Mean Square Error = 78794.63
Critical Value = 2.10
Least Significant Difference = 481.52

The grouping letters indicate that the product mean values (group 3) at day 7 and day 28 are significantly different from those of both the nutrient control (group 2) and the non-nutrient control (group 1) for those days. No other significant differences are shown. Therefore, in terms of total aromatic degradation, the test indicates the desired statistically significant difference between the mean of the product and the mean of the non-nutrient control.

EXPERIMENTAL DESIGN

The shaker flask evaluation conducted in Tier II is an experiment designed to determine the product's ability to degrade crude oil components at a rate or extent greater than a natural seawater microbial population. The experimental design includes a control, nutrient treatment, and the product treatment. The resultant data are compared and tested statistically using a two-way analysis of variance to determine data trends. The experimental design for Tier II testing is known as a factorial experiment with two factors. The first factor is product/control group; the second factor is time (as measured in days). For example, if two groups (product A and a non-nutrient control) are tested at each of three points in time (day 0, 7, and 28), the experiment is called a 2x3 factorial experiment. There were three replications (replicated shaker flasks) of each group-time combination.

DATA ANALYSIS METHODS

For each analyte and each product used in Tier II, a product is deemed a success by the demonstration of a statistically significant difference between the mean analyte degradation by the product and the mean analyte degradation by the non-nutrient control. Such a determination will be made by performing a two-way analysis of variance (ANOVA) on the sample data. The technical aspects of this procedure are outlined in Snedecor and Cochran (1980). Most statistical software packages support the use of two-way ANOVA. However, the format required for the input data differs among the various commercial packages. Whichever package is used, the following ANOVA table will be provided as part of the output.

TABLE 1
TWO WAY ANOVA TABLE

Source	df	Sum of Squares	Mean Square	F-statistic	p-value
Group	p-1	SSG	MSG = MSG/MSE	MSG/MSE	*
Time	t-1	SST	MST = MST/MSE	MST/MSE	*
Interaction	(p-1)(t-1)	SSI	MSI = MSI/MSE	MSI/MSE	*
Error	pt(n-1)	SSE	MSE = SSE		
TOTAL	npt-1	SSTOT			

* To be determined from the value of the F-statistic

In the degrees of freedom column (df) of Table 1, **p** denotes the number of product/control groups, **t** denotes the number of days at which each group is analyzed and **n** denotes the number of replications. For the example of the 2x3 factorial experiment discussed in the previous section, $p = 2$, $t = 3$, and $n = 3$. The significance of the F-statistics (as indicated by their corresponding p-value) are used to interpret the analysis.

INTERPRETATION

If the F-statistic for the INTERACTION is significant at the 0.05 level (i.e. the p-value is less than 0.05), the data indicate that the mean response of at least two groups being tested differ for at least one point in time. In order to find out which groups and at which points in time the difference occurs, pairwise comparisons between the group means should be conducted for all time points. These comparisons can be made using protected least squared difference (LSD) or Dunnett mean separation techniques. The protected LSD procedure is detailed in Snedecor and Cochran (1980); the Dunnett procedure is outlined in Montgomery (1991). For both methods, the mean square error (MSE) from the two-way ANOVA table should be used to compute the separation values.

If the F-statistic for the INTERACTION is not significant at the 0.05 level (i.e. the p-value is not less than 0.05), but the F-statistic for the GROUP is significant (i.e. the p-value is less than 0.05), the data indicate that any differences which exist among the group means are consistent across time. To find out which group means differ, a pairwise comparison of the group means should be carried out by pooling data across all points in time. Again, the mean square error (MSE) from the two-way ANOVA table should be used to compute the separation values.

If the F-statistic corresponding to both INTERACTION and GROUP are not significant at the 0.05 level, the data indicate no difference between the group means at any point in time. In this case, no further analysis is necessary.

Finally, Snedecor and Cochran (1980) caution about the use of multiple comparisons. If many such comparisons are being conducted, then about 5 percent of the tested differences will erroneously be concluded as significant. The researcher must guard against such differences causing undue attention.

REQUIRED DOCUMENTATION

The following documents should be included to summarize findings from a product test.

- Data listings for each analyte that was analyzed. These should show all raw data.
- A table of summary statistics for each analyte. The table should include the mean, standard deviation and sample size for each group at each day.
- An ANOVA table for each analyte. The table should be of the same format as Table 1
- A clear summary of the mean separations (if mean separations were necessary). The mean separation methods (LSD or Dunnett), the significance level, the minimum significant difference value and the significant differences should be clearly marked on each output page.
- All computer outputs should be included. No programming alterations are necessary. The specific computer package used to analyze the data should be included in the report.

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OIL SPILL EATER II
EPA TEST - MARCH 1993
OIL SPILL EATER II - RESPIROCITY TEST - SUMMARY

This Respirocity Test was developed by NETAC and the Environmental Protection Agency to verify if a product could actually mitigate hydrocarbons to an end point of C02 and water. The test was designed to measure the amount of oxygen-enhanced bacteria used. This would confirm the bacteria are in fact breaking the hydrocarbons down to C02 and water.

At 100 parts Alaskan Gulf Seawater to 1 part OIL SPILL EATER II - applied at a 1 to 1 ratio to 1,000 parts per million Alaskan Prudhoe Bay Crude, the oxygen uptake is dramatic. This dramatic oxygen uptake proves a large amount of bacterial growth and decomposition of Prudhoe Bay Crude. The Chart on Page 2 shows an 86% decrease in heavy-end hydrocarbons and a 50% decrease in the aromatics. The test was stopped at 30 days; the test time prescribed by the EPA.

Our Standard Application Instructions for crude oil are 50 parts water to 1 part OIL SPILL EATER II applied at a 1 to 1 ratio to crude oil. The test results may be extrapolated to determine that with a 50 to 1 dilution, a 98% decrease in heavy-ends would occur in 24 days while an 85% decrease in aromatics would occur in 30 day. aeromatics would occur in 30 days. OIL SPILL EATER II can very effectively mitigate an oil spill.

After reviewing copies of the EPA Test on 10 other products, a comparison was initiated on the 2 products EPA claimed out-performed the other 9 products they tested. One product reduced the TPH approximately 158 parts per million and the other product reduced to 157 ppm. of TPH. OIL SPILL EATER II reduced the TPH to 870 PPM. We feel this is a significant difference in efficacy.

March 1993
Respirocity Test

The Prudhoe Crude was supplied by the EPA and was supposed to be the same crude used on the other two products. The crude sent to us for testing had a higher TPH (1,000 PPM.) compared to the bacteria products tested by the EPA which only had a TPH of 168 ppm. Additionally, this crude did not have aromatics which the crude oil OSE II was tested on, did. The aromatics were reduced 50%.

It is our opinion that if you apply bacteria directly to a hydrocarbon with aromatics, that the toxicity of the aromatics will kill the bacteria. OIL SPILL EATER II first breaks the hydrocarbon walls, then grows bacteria so the toxicity is reduced first.

The accumulate oxygen uptake was also tested which shows bacterial activity. One of the products the EPA tested, they claim, performed well, had an uptake of 280 mg/L in 10 days and 460 mg/L in 30 days. The other product the EPA tested had 40 mg/L at 10 days and 440 mg/L at 30 days. OIL SPILL EATER II had an uptake of 520 mg/L at 10 days and 810 mg/L at 30 days. OSE II had more oxygen uptake at 10 days than the best bacterial products had at 30 days; on the 30 day comparison, OSE II had almost double the oxygen uptake as any other product.

The EPA screened 31 products and tested 10. This test shows OIL SPILL EATER II reduced dramatically more TPH than these other products. OSE II produces more microbial activity than products with bacteria, and additionally, OSE II reduces aeromatics. This test should help prove why we feel OSE II is the better product.

NOTE: In the summer of 2000 - Dr. Al Venosa (one of the EPA's top scientists at the time, on oil spills) reviewed this test. Dr. Venosa concluded that OSE II did, in fact biodegrade alkanes and aromatics. Dr. Venosa went on to explain that OSE II may be effective in degrading oil.

Steve Pedigo

By: Steven R. Pedigo
Chairman
OSEI, Corp.

SRP/AJL

CHEMICAL ANALYSIS, INC.

Chemical & Polymer & Design

Research and Development
Consultation
Legal and Expert Witness

July 3, 1990

Failure Analysis
Formula Analysis
Engineering Design

Mr. Steve Pedigo
Sky Blue Chems
13355 Noel Road
1 Galleria Tower, Suite 500
Dallas, Texas 75240

NEW ADDRESS AS OF 10/96

OSEI, CORP.
13127 Chandler Drive
Dallas, TX 75243

Subject: Oil Spill Eater Respirocity Evaluation
CAI Lab. No. 3265

Dear Mr. Pedigo:

Chemical Analysis, Inc. being an independent third party laboratory was employed to evaluate an oil spill additive for respirocity efficacy. The oil spill additive submitted to the laboratory was a product identified as Oil Spill Eater batch No. 124-E. The additive was evaluated at two different concentrations which included 1/100 and 1/500, additive parts to solution parts, respectively.

The concentration of the oil was 1000 parts per million (ppm). The oil and seawater was submitted to the laboratory to be similar to field material.

The results of our evaluation are attached to the report. Observing the results, it can be seen that the additive has a meaningful and significant effect on decreasing the oil concentration and increasing the oxygen take up.

The effect on decreasing the aliphatic content of the oil was in the range of 80 percent and the decrease of the aromatic content was in the range of 40 percent. An additive concentration of 1/500 appears to be effective. The concentration of the additive may have an adequate effect at even a lower concentration than 1/500.

The inherent effect of oxygen takeup was observed to be 178 mg/L for the additive (1/500), 12 for the seawater, and 8 for the oil. The net effect of the additive was 512 mg/L.

If there are any questions or if we may be of further assistance, please advise.

Sincerely yours,
CHEMICAL ANALYSIS, INC.

Galen Hartman
Laboratory Director
GWH:es

Oil Spill Eater (OSE) Respirocity Results

				Accumulated Oxygen Uptake				Aliphatic Content				Aromatic Content		Percent	Percent
mple	Oil	Additive	Seawater	0 mg/L	10 mg/L	20 mg/L	30 days mg/L	0 ppm	10 ppm	20 ppm	30 days ppm	0 ppm	30 days ppm	Aliphatic Decrease	Aromatic Decrease
1	+	1/500	+	16	380	620	690	712	570	233	151	246	133	79	46
2	+	1/500	+	18	410	660	730	693	542	274	138	240	149	80	38
3	-	1/500	+	5	152	174	186	-	-	-	-	-	-	-	-
4	-	1/500	+	5	141	168	194	-	-	-	-	-	-	-	-
5	-	-	+	0	5	8	12	-	-	-	-	-	-	-	-
6	-	-	+	0	6	8	11	-	-	-	-	-	-	-	-
7	+	-	+	2	12	18	22	705	710	695	682	251	248	3	1
8	+	-	+	3	13	16	19	684	680	681	675	238	237	1	0
9	+	1/100	+	26	460	680	770	690	512	827 210	105	245	115	85	53
10	+	1/100	+	33	520	740	810	695	486	260	89	250	127	87	49

Oil Spill Eater Batch No. 124-E

OIL SPILL EATER INTERNATIONAL, CORP.

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March 23, 1990

OIL SPILL EATER II
BIODEGRADATION TESTS
CONCLUSIONS

These tests were conducted by the University of Alaska in Fairbanks, AK. The first test was on a heavy-end hydrocarbon (Hexadecane), which is left over once the light-ends volatize off. The mineral nutrients in nature refers to the use of Alaskan Sea Water used to perform the test. At 50 to 1, it shows good reduction and if the test would have continued another 48 hours, the results would have been substantially increased. The OIL SPILL EATER II has a good food source for bacteria and there was more food source than sea water ratio to grow a large colony quickly; therefore, the bacteria engulfed the food sources in the OSE II and slowly converted to hydrocarbons. Once all the OSE II food source runs out, then the only food source left are the hydrocarbons - so they switch over to stay alive. At 1 to 500 and 1 to 1000 absolute biodegradation was proven, the bacteria colonized quickly and ran out of food source because they started with less food source. The bacteria switched over quickly and a dramatic reduction in hexadecane was accomplished.

The second test was run on Napthalene using minerals and nutrients (Alaskan Sea Water). Naphthalene is a polynuclear aeromatic hydrocarbon and are harder to break down than heavy-end hydrocarbons and they are the most toxic. These tests also show that OIL SPILL EATER II is a very effective means of mitigating naphthalene, a PAH which EPA's Dr. Al Venosa deems the hardest target compounds to Bioremediate!

Steven Pedigo

By: Steven R Pedigo
Chairman

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OIL SPILL EATER II

A PROTEIN POWER PACKAGE

The lack of knowledge about biological treatment of hydrocarbons has laid to slow acceptance of proven methods of Bioremediation, particularly with respect to oil spills. However, following the EXXON VALDEZ incident, the U. S. Environmental Protection Agency undertook the first major governmental effort to use biological methods for site remediation. Although the early results are mixed, EPA is to be commended for its efforts which included application of a French Product (Inipol EPA 22) to enhance microbial degrading of weathered crude oil from beaches. Inipol has been described as a "Popeye's Spinach" supplement to enhance the rate and extent of hydrocarbon degradation by naturally occurring microbial populations. The Inipol formulation probably does enhance the growth of hydrocarbon degradation bacteria (although this has not been clearly shown in the field portion of the EPA Study), but suffers in that it contains the potentially toxic solvent, 2-butoxyethanol.
EPA Study),

There are many other agents which have potential to stimulate hydrocarbon removal from contaminated environments. These range from the solvent based cleaners and dispersants to simple water soluble inorganic fertilizers. One such product that has shown great potential for enhancing hydrocarbon biodegadation in standardized laboratory tests at the University of Alaska Fairbanks, is OIL SPILL EATER II. If Inipol is a "Popeye's Spinach" formulation for hydrocarbon degrading micro-organisms, OIL SPILL EATER II is a "Protein Power Package" of mineral nutrients, enymes and a carbon source concentrated in a non-toxic oleophilic surfactant. The surfactant base disolves into hydrocarbon matrices with the aid of protease and amylase enzymes that act as micro-surface cleaners. The mineral nutrients enhance growth of natural hydrocarbon degrading micro-organisms with the pulse of easily metabolized carbon to quickly increase bio-mass. The high bio-mass, then begins to degrade hydrocarbon substrates and to product biosurfactants until the hydrocarbon substrate is depleted.

OIL SPILL EATER II
A PROTEIN POWER PACKAGE

In the aftermath of the EXXON VALDEZ Oil Spill, researchers from the University of Alaska evaluated the potential for naturally occurring micro-organisms to biodegrade oil contaminated beaches. Their studies showed that while natural micro-organisms have the potential to biodegrade both linear alkanes and aromatic hydrocarbons, their numbers and related metabolic activities can be substantially increased. In standard laboratory tests, these researchers showed that the marine formulation of OIL SPILL EATER II diluted into artificial seawater containing a consortium of micro-organisms and hydrocarbons from Prince William Sound, Alaska will degrade Hexadecane - 300 % faster than the same consortium amended with mineral nutrients and hydrocarbons without OIL SPILL EATER II.

By: Dr. Ed Brown
University of Alaska

DEB/AJL

OIL SPILL EATER CONCENTRATE

MINERALIZATION OF HEXADECANE BY A MICROBIAL CONSORTIUS FROM PRINCE WILLIAM SOUND, ALASKA (1)

Sample	Mineral Nutrients in nature HO OSE	Mineral Nutrients in nature 1/50 Dilution of Oil Spill Eater II	Mineral Nutrients in nature 1/500 Dilution of Oil Spill Eater II	Mineral Nutrients in nature 1/1000 Dilution of Oil Spill Eater II	Mineral Nutrients in nature 1/10 Dilution of Oil Spill Eater II
Hexadecane Transformation (I transformed to CO2) Mean of 3 trials	16	19.3	50	43.7	0

Need more time so bacteria can use up mollases & convert to Hydrocarbon

300 increase

proven efficacy

Should totally eliminate Hydrocarbons

1. Consortius was incubated for 70 hours with 100 mg of labeled hexadecane per sample.

Test Conducted at University of Alaska-Fairbanks

OIL SPILL EATER II CONCENTRATE
Mineralization of Naphthalene by a Microbial Consortium From Prince William Sound, Alaska (1)
Alaskan Seawater

Sample	MINERAL Nutrients in nature No OSE	MINERAL Nutrients in nature 1/50 Dilution of Oil Spill Eater II	MINERAL Nutrients in nature 1/500 Dilution of Oil Spill Eater II	MINERAL Nutrients in nature 1/1000 Dilution of Oil Spill Eater II
N A P H T H A L E N E Transformation (% transformed to CO_2 Mean of 3 trials	3	29	46	27

More time would have been allowed for the bacteria to completely use up the molasses and completely convert to hydrocarbon for its food source

1 5 3 3 % increase

p r o v e n efficacy

s h o u l d t o t a l l y eliminate raphthalene hydrocarbons

1. Consortium (Alaska Sea Water) was incubated for 51 hours with 100 Ug of labeled Naphthalene per 10 ML sample.

Test conducted at the University of Alaska
1/9/90

OIL SPILL EATER INTERNATIONAL, CORP.

13127 Chandler Drive
Dallas, Texas 75243
(972) 669-3390
(972) 644-8359 (Fax)

August 13, 1990

MEGA BORG BIODEGRADATION TEST

Southwest Research Institute - one of the United States largest and most respected labs performed TPH reduction tests and residual weight tests using OIL SPILL EATER. This product, OSE, was applied to South African Crude Oil - spilled from the Mega Borg Tanker off the coast of Galveston, Texas. The sample of crude was supplied by the U. S. Coast Guard - Sky Blue Chems sent the sea water from Galveston to the Lab.

The initial TPH was 100,070 ppm; in 216 hours the TPH was reduced to 529 for a 99.5% reduction. This is a dramatic decrease and it proves Oil Spill Eater is a very viable Bioremediation product. This dramatic decrease shows how effective Oil Spill Eater is in reducing the chemical (toxic) constituent of the crude oil. The TPH was reduced approximately 90% in 48 hours rendering the crude oil virtually harmless quickly.

The physical reduction of the crude oil was also determined. In 216 hours, 94.7 of the residual weight of the South African Crude was Bioremediated.

These tests prove "OIL SPILL EATER" is an extremely effective Bioremediation product that decreases not only the chemical components of crude oil, but it also Biodegrades the physical components as well.

Steven R. Pedigo
Chairman

SRP/AJL

SOUTHWEST RESEARCH INSTITUTE

6220 CULEBRA ROAD • POST OFFICE DRAWER 28510 • SAN ANTONIO, TEXAS 78228-0510 • (512) 684-5111 • TELEX 244846

CHEMISTRY AND CHEMICAL ENGINEERING DIVISION
DEPARTMENT OF ENVIRONMENTAL SCIENCES

August 3, 1990

Attention: Mr. Steven R. Pedigo

Subject: Second Test for Sky Blue Chemical 01-3108-092

A sample of Megaborg oil and seawater was analyzed as per your instructions. The results of this initial test were inconclusive and a second test was requested. The second test was more extensive and included more time points. Samples were taken at 48,72, and 96 hours for the sample and control. The sample consisted of 600 ml seawater, 6 ml Megaborg oil, and 6 ml of the oil-eater provided. The control consisted of 600 ml seawater, and 6 ml Megaborg oil. The sample and control were stirred constantly at a very low speed. Sampling procedure: Vigorously stir the solution and remove 100 ml. Extract for TRPH analysis. After, 90 hours the client requested addition of more seawater to improve the efficiency of the oil-eater, this was performed. A final analysis for TRPH was performed at 216 hours and was a complete sample extraction. In order to better compare the control and oil-eater results, results are shown in % Recoverable Oil, assuming that 1 gram of oil is equal to 1 ml of oil (since oil density is unknown). The percent recoverable oil is calculated as follows:

$$\frac{\text{TRPH } \mu g/ml \times \frac{100 \text{ ml}}{1000 \ \mu g/mg}}{\text{theoretical amount of oil extracted in each aliquot} = 1 \text{ g}} \times \frac{100}{1000 \text{ mg/g}} = \%$$

TRPH and % Recoverable Oil for each time are shown for the sample and control in tables 1 and 2, respectively. Megaborg oil itself was found to have a TRPH of 1,070,000 mg/l.

Sincerely,

Mary Riddle

Mary Riddle
Research Scientist

Approved:

Donald E. Johnson

Donald E. Johnson, Ph.D.
Director

SAN ANTONIO TEXAS
DALLAS FT. WORTH TEXAS • HOUSTON TEXAS • DETROIT MICHIGAN • WASHINGTON DC

Table 1

01-3108-092
Sample with Oil-Eater II

Time Elapsed	TRPH(mg/10)	%Recoverable Oil
48 hours	7520	75.2
72 hours	6910	69.1
96 hours	5990	59.9
*216 hours	529	5.3

95% Reduction of TPH in 216 hours. Chemical reduction of TPH.

94.7% residual weight reduction in 216 hours. Physical reduction of oil.

* - Total sample analyzed

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SUMMARY OF BETX TEST

The objective was to have a third party testing laboratory show how OSE II (OIL SPILL EATER II Concentrate) worked well even on Benzene, Ethyl Benezene, Toulene and Xylene. The final composition - after all dilutions were performed, was 2,000 parts water to one (1) part OSE II Concentrate.

Even at this low level, the total BETX was reduced 32%. The correlation of strengths should prove that at 1,000 to one (1) reduction would have been 64%, a 500 to one (1) reduction would have been 80%; a 100 to one (1) reduction would have been 98%, almost completely Biodegraded.

At 2,000 to one (1) OSE II is a cost- effective product for Ballast Water Treatment.

The reduction correlation's with the increasing ratios also show that OSE II is an effective product for gasoline and diesel spills. OSE II would reduce gasoline or diesel spills on the surface and around leaking Underground Storage Tanks. OSE II would also be a good product to clean up any oil sheen on water surfaces and concrete surfaces.

Steven R. Pedigo
Chairman

CHEMICAL ANALYSIS, INC.

Chemical ⁂ Polymer ⁂ Design

Research and Development
Consultation
Legal and Expert Witness

Failure Analysis
Formula Analysis
Engineering Design

March 14, 1990

Mr. Steve Pedigo

Subject: BETX Analysis
CAI LAB NO.: 3229

Reference: Oil Spill Eater Evaluation

Dear Mr. Pedigo:

Chemical Analysis, Inc. being a third party independent laboratory was employed to evaluate a product identified as Oil Spill Eater and its affect on BETX solution. The procedural method was provided to our laboratory which outlined the preparation of several solutions.

Solution I: BETX

COMPONENTS	% BY VOLUME
Benzene	5.0
Ethylbenzene	5.0
Toluene	5.0
Xylene	5.0
Florida Sea Water	80.0
TOTAL	100.0 %

Solution II: OSE-Florida Sea Water

COMPONENTS	% BY VOLUME
Oil Spill Eater	0.20
Florida Sea Water	99.80
TOTAL	100.00 %

The percentage ratio of these two components represents a 1 to 500 mix ratio respectively.

3001 Skyway Circle North, Suite 100, Las Colinas ⁂ Irving, Texas 75038 ⁂ (214) 255-4100

Solution III: BETX/OSE-Florida Sea Water

COMPONENTS	% BY VOLUME
Solution I	50.0
Solution II	50.0
TOTAL	100.0 %

Solution IV: BETX/OSE-Florida Sea Water Solution

COMPONENTS	% BY VOLUME
Solution III	50.0
Florida Sea Water	50.0
TOTAL	100.0 %

Final Solution Composition:

COMPONENTS	% BY VOLUME
Aromatics	5.0
OSE Additive	0.05 (1:2000 weight ratio)
Florida Sea Water	94.95
TOTAL	100.00 %

The final solution identifies the composition of the final mixture when the various solutions are prepared and mixed together based on the procedural instructions. The resultant final solution was allowed to stir for a period of (96) hours and the volume of BETX aromatic content was evaluated. The initial percent volume of aromatic discontinuous phase in the final solution represented five percent after the test. As a result of the evaluation, it was observed that 1.6% of the BETX solution had decreased from the discontinuous aromatic phase; this represented a 32% volume reduction in the aromatic content. Turbidity was observed to have increased in the water phase which indicated that incompatable components were incorporated into the water phase.

The 1:2000 weight ratio concentration of OSE in the final solution is based on the assumption that the OSE additive is 100% active; if the OSE is less than 100% active then one needs to porportionate the concentrtion accordingly.

If there are any questions or if we can be of any further assistance, please advise.

Sincerely yours,

CHEMICAL ANALYSIS, INC.

Galen W. Hartman
Laboratory Director

GWH/cmc

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SUMMARY

CHEVRON CRUDE OIL TEST

A client of OSEII requested that we perform a basic test on Chevron crude oil to show the potential for OSEII to bioremediate this oil.

A basic test where crude oil was placed on water and OSEII was applied was performed. The initial TPH count was 95,200 ppm. OSEII was applied on 1-18-91. The next test was performed 12 days later where the TPH had dropped to 7,720 ppm. Then 12 days later, the final test was performed and the TPH had dropped to 690 ppm.

This was a simple test to show the client that indeed OSEII would remediate the type of contamination on their site.

Steven Pedigo
Chairman

NATIONAL ENVIRONMENTAL TESTING, INC.

NET Gulf Coast, Inc.
Dallas Division
1548 Valwood Parkway
Suite 118
Carrollton, TX 75006
Tel: (214) 406-8100
Fax: (214) 484-2969

Mailing Address:
P.O. Box 815006
Dallas, TX 75381

ANALYTICAL REPORT

OSE
5545 Harvest Hill Lane
Suite 1116
Dallas, TX 75230

02-04-91
Job No.: 903119
Sample No: 157555-157556
Page: 1

Sample Description: SEE BELOW

Date Received: 01-18-91

157555 Chevron Crude - Sherman TX
Taken: 01-18-91

Total Petroleum Hydrocarbon 952,000* ug/g x density 95,200*

157556(1) Chevron Crude - Remediation Treated
Taken: 01-18-91

Total Petroleum Hydrocarbon 77,100* ug/g x density 7,720*

On January 30, 1991 sample was mixed and total TPH analyzed.

157556(2) Chevron Crude - Remediation Treated
Analyzed 2/12/91

Total Petroleum Hydrocarbon 6,900* ug/g x density 690*

On February 12, 1991 sample was mixed and total TPH was analyzed.

* Freon Extract Discolored.

Donna L. Bowlin KSD

Donna L. Bowlin, Manager
Dallas Division

STANDARD QUALITY CONTROL DATA REPORT

SAMPLE/PROJECT 157555-157556

PARAMETER	ANALYST	DATE	TIME	METHOD	EXTERNAL STANDARD	BLANK
TPH	DWT	013091	1000	E418.1	1880/1700	BDL
TPH	DWT	021291	1000	E418.1	2270/2440	BDL

Method - Codes, i.e.
- A - refers to APHA, Standard Methods for the Examination of Water and Wastewater, 16th edition
- E - refers to EPA's 1979 Methods for Chemical Analysis of Water and Wastes - for Inorganic Analyses
- E - refers to EPA's 1979 Methods for Organic Chemical Analysis of Municipal and Industrial Wastes - for Organic Analyses
- S - refers to SW846, 3rd edition
- D - refers to ASTM
- M - Method has been modified
- * - refers to Other Reference

External Standard - the Actual/Theoretical value for that batch of analysis. Acceptance Criteria - must be within 10% of the true value, except where EPA methods state otherwise.
Blank - samples are not blank corrected by the laboratory

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"OIL SPILL EATER II"

HYDROCARBON REDUCTION TEST

FOR
GAF INDUSTRIES

SUMMARY

GAF Industries in Savannah, Georgia has a site contaminated with Venezuelan crude, #6 fuel oil and diesel fuel. The site has been contaminated for approximately 10 years. Sky Blue Chems designed a lab test that would mimic the actual cleanup plan. The contaminated site had approximately 85% aliphatic (heavy end) hydrocarbons, 6% aromatics (light ends) and 9% asphaltenes (weathered crude).

The initial hydrocarbon count was 100,000 mg/L. Oil Spill Eater II was mixed 50 to 1 with Savannah river water and applied at a 1 to 1 ratio to the hydrocarbons. In 96 hours all the aromatics and all the aliphatics were reduced to CO_2 and water. The weathered asphaltenes were the hardest to breakdown and consumed most of the testing time.

GAF asked us to demonstrate that we could mitigate their hydrocarbon contamination to less than 100 ppm so they could meet their NPDES discharge permit needs. This was a rigorous test for Oil Spill Eater II that proves the product is effective on light ends, heavy ends and weathered asphaltenes.

Steven Pedigo
Chairman

SL SAVANNAH LABORATORIES
& ENVIRONMENTAL SERVICES, INC.

5102 LaRoche Avenue • Savannah, GA 31404 • (912) 354-7858 • Fax (912) 352-0165

LOG NO: S0-06430

Received: 24 MAY 90

CC: Pedigo/Franklin

REPORT OF RESULTS Page 1

LOG NO	SAMPLE DESCRIPTION , SOLID OR SEMISOLID SAMPLES	SAMPLED BY
06430-1	GAF Waste Comp. Initial Test 6/1/90	Savannah Laboratories

PARAMETER	06430-1
Petroleum Hydrocarbons (418.1), mg/kg	100000
Percent Solids, %	56 %

CC: Pedigo/Franklin

REPORT OF RESULTS Page 2

LOG NO	SAMPLE DESCRIPTION , LIQUID SAMPLES	SAMPLED BY
06430-2	GAF Waste Composite Second Test 6/8/90	Savannah Laboratories
06430-3	GAF Waste Composite Third Test 6/11/90	
06430-4	GAF Waste Composite Fourth Test 6/15/90	

PARAMETER	06430-2	06430-3	06430-4
Petroleum Hydrocarbons (418.1), mg/l	6800	5400	5000

SAVANNAH LABORATORIES
& ENVIRONMENTAL SERVICES, INC.

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LOG NO: S0-06430

Received: 24 MAY 90

CC: Pedigo/Franklin

REPORT OF RESULTS

LOG NO	SAMPLE DESCRIPTION , LIQUID SAMPLES	SAMPLED BY
06430-5	GAF Waste Composite Fifth Test 6/22/90	Savannah Laboratories
06430-6	GAF Waste Composite Sixth Test 6/26/90	
06430-7	GAF Waste Composite Seventh Test 6/29/90	
06430-8	GAF Waste Composite Eighth Test 7/3/90	
06430-9	GAF Waste Composite Ninth Test 7/6/90	

PARAMETER	06430-5	06430-6	06430-7	06430-8	06430-9
Petroleum Hydrocarbons (418.1), mg/l	2800	990	1500	1500	1100

Methods: 1) EPA SW-846.
2) Sky Blue Chem Procedure "Testing Proposal OSE Bioremediation of Hydrocarbons".

Note: Extraction protocol described in Method 2 followed. Verbal instructions received on 6/22/90 to maintain volume by replacing each 100 ml aliquot removed for analysis with 100 ml of river water. A total volume of 500 ml OSE was added in seven applications.

SL SAVANNAH LABORATORIES
& ENVIRONMENTAL SERVICES, INC.

5102 LaRoche Avenue • Savannah, GA 31404 • (912) 354-7858 • Fax (912) 352-0165

LOG NO: S0-06430

Received: 24 MAY 90

CC: Pedigo/Franklin

REPORT OF RESULTS

LOG NO	SAMPLE DESCRIPTION , LIQUID SAMPLES	SAMPLED BY
06430-10	GAF Waste Composite Tenth Test 7/10/90	Savannah Laboratories
06430-11	GAF Waste Comoposite Eleventh Test 7/13/90	
06430-12	GAF Waste Composite Twelfth Test 7/17/90	
06430-13	GAF Waste Composite Thirteenth Test 7/20/90	

PARAMETER	06430-10	06430-11	06430-12	06430-13
Petroleum Hydrocarbons (418.1), mg/l	700	350	360	41

Methods: 1) EPA SW-846.
2) Sky Blue Chem Procedure "Testing Proposal OSE Bioremediation of Hydrocarbons".

Note: Extraction protocol described in Method 2 followed. Verbal instructions received on 6/22/90 to maintain volume by replacing each 100 ml aliquot removed for analysis with 100 ml of river water. A total volume of 500 ml OSE was added in seven applications.

William D. Sherrod

William D. Sherrod

OIL SPILL EATER INTERNATIONAL, CORP.

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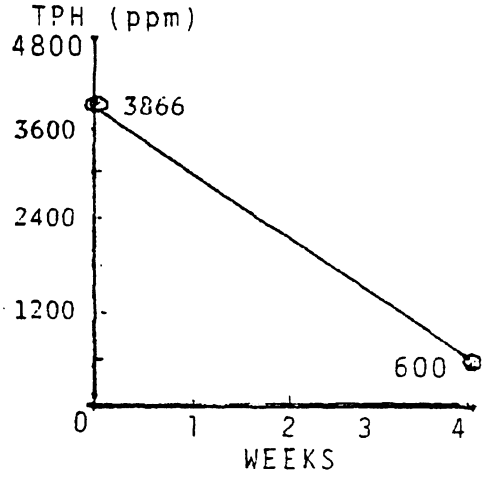
DIESEL CONTAMINATION CLEANUP SUMMARY 27 July 1993
U. S. Marine Corps, 29 Palms, CA

CONTAMINANT: 89 cubic yards of aged Diesel Contaminated Soil. The soil was spread out in a rectangle approximately 33 inches deep. NOTE: Our instructions specify no deeper than 18 inches.

PROCEDURE: 29 Palms initial composite sample was 3,986 ppm TPH using EPA Test 8015 modified. 29 Palms applied 40 gallons of OSE II mixed with 2,000 gallons of pond water to the contaminated soil. They used a tanker truck and fire hose to apply the liquid.



29 Palms did not disc or add any additional water to the contaminated soil for the next four (4) weeks. NOTE: Our instructions specify to disc the soil at least once a week and keep the soil at a level of approximately 30% humidity.

29 Palms took samples after 4 weeks and had a composite reading of 600 ppm TPH. THE ACCEPTABLE LEVEL IS 1,000 ppm TPH.

COMMENT: EVEN THOUGH 29 PALMS . . .

1. Piled the contaminated soil almost twice as deep as our instructions specify, and

2. Did not disc the contaminated soil at all during the 4 weeks, and

3. Did not add additional water to the contaminated soil in the desert climate -

   "OIL SPILL EATER" II W O R K E D!!

4. Think of what the TPH level might have been had 29 Palms followed our OSEI Procedures?

O. A. (George) Lively
Rear Admiral (RET)
President

UNITED STATES MARINE CORPS
NATURAL RESOURCES and ENVIRONMENTAL AFFAIRS
Marine Corps Air Ground Combat Center
Box 788110
Twentynine Palms, California 92278-8110

5090
9
15 Oct 93

MEMORANDUM

From: Installation Restoration (IR) Specialist
TO: FILE

SUBJ: TESTING AND EVALUATION OF ENZYMATIC CATALYSIS FOR THE REMEDIATION OF PETROLEUM CONTAMINATED SOILS.

1. On 20 July 1993, MCAGCC began a testing and evaluation demonstration of a commercially available product of a natural biological enzymatic catalyst for the remediation of petroleum contaminated soils. The product, Oil Spill Eater II (OSE II), is on the National Contingency Plan Product Schedule of Biological Additives and is authorized for use by On-Scene Coordinators on releases of petroleum oil.

2. Before the Combat Center deploys this additive on a petroleum spill site or in the remediation of soils resulting from a spill, the Command decided to try the product on a small scale for it's effectiveness. A summary of the results are as follows:

   . A control pile was constructed by berming and double lining (2 - 40 mil thick HDPE Liners) of a test area 21 feet X 42 feet. Non-hazardous soils under 40 CFR or Title 22 of CCR was placed in a 2' 9" high lift on top of the liner; resulting in approximately 69 cubic yards of petroleum contaminated soils from oil/water separators (OWS) to be tested with the product.

   . Three soil samples were obtained on 26 July 93 within the pile and were sent to on off-site laboratory for the analysis of BETX by EPA 8020. Total Fuel Hydrocarbon by EPA 8015 (modified) as Diesel and Organic Lead by DHS Method.

   . On 28 July 1993, the test product was mixed according to manufacture application specifications of 40 gallons of product to 2,400 gallons of water. Application over the pile was accomplished by spraying with a 1,000 gallon water truck equipped with a 50 gpm pump. The pile was then covered with a black 12 mil thick plastic.

   . On 30 August 1993, three additional samples were taken to check progress of remediation. The samples were sent to an offsite laboratory for the same anaylsis of the initial sampling event. Due to lack of manpower, sampling could not begin being conducted at a two week interval.

3. The results are as of the testing as follows:

INITIAL SAMPLING EVENT OF 26 July 93 (1)

	Sample Number	OS-1	OS-2	OS-3
Analyte	Reporting Limit mg/kg	Concentration mg/kg		
Benzene	0.005	<0.05	<0.05	<0.05*
Toluene	0.005	<0.05	<0.05	0.77
Ethylbenzene	0.005	<0.05	<0.05	0.26
Xylene, total	0.015	<0.15	<0.15	1.6
BTEX, total	---	---	---	2.6
TPH as diesel	10	2000	1400	8200
Organic Lead**	0.5	0.5	0.5	1.2

SAMPLING EVENT OF 30 AUGUST 1993

	Sample Number	OS1-A*	OS1-B*	OS1-C*
Analyte	Reporting Limit mg/kg	Concentration mg/kg		
Benzene	0.005	<0.05	<0.05	<0.05
Toluene	0.005	<0.05	<0.05	<0.05
Ethylbenzene	0.005	<0.05	<0.05	<0.05
Xylene, total	0.015	<0.15	<0.15	<0.15
BTEX, total	---	---	---	---
TPH as diesel	10	820	380	600
Organic Lead	0.5	<0.5	<0.5	<0.5

(1) Holding time was missed due to shipping of initial sampling delays.
* Reporting limit raised due to matrix effect (foaming).
** Extraction by DHS Method. Results are calculated on a wet weight basis. Total Organic Lead in Soil by Flame AA - DHS.

Subj: TESTING AND EVALUATION OF ENZYMATIC CATALYSIS FOR THE REMEDIATION OF PETROLEUM CONTAMINATED SOILS

4. It is assumed the reason for the matrix foaming of the samples are due to surfactants in the soil and from the test product.

5. On the 30 August 1993 sampling event, soil samples were moist to saturated from the application of product. No tilling or turning of soils were required as recommended by the contractor.

6. Results indicate bioremediation of soils within acceptable levels required by the Regional Water Quality Control Board (RWQCB) for the soils to be used as landfill cover.

7. A request for disposal of remediated soils at the MCAGCC Class III landfill will be forwarded to the RWQCB.

8. Additional, test piles will be run in the future utilizing OWS, JP-5 and diesel contaminated soils based on the availability of manpower.

LEON BOWLING

OIL SPILL EATER INTERNATIONAL, CORP.

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"OIL SPILL EATER II"

SOIL TPH TEST

CONCLUSION

The contaminated soil was extracted from a site in Anchorage, Alaska. The initial total parts hydrocarbon (TPH) was 767 and after three (3) diluted applications, applied per oil spill instructions for soil contamination cleanup, the TPH reduced to 391. This soil had been contaminated for approximately one (1) year which means the contaminant had weathered substantially. The more weathered, the more resistant the hydrocarbons are to remediation, which takes slightly longer for OSEII to help remediate the TPH. The test was carried out using sterile deionized water, which means bacteria take longer to colonize. If natural sea water or fresh water with naturally occurring bacteria already in it had been used, biodegradation would have been more rapid and a higher reduction of TPH would have occurred.

One of the concerns is once OSEII is applied, did bioremediation actually occur, or were the hydrocarbons simply mobilized into the water column (aqueous phase)? A test on the Supernate of the treated soil (the water used in the test) was then tested for its hydrocarbon content. The test showed only 2% hydrocarbon count which could be expected since the water is covering the contaminated soil. It does, however, definitely prove that the hydrocarbons are being converted to CO_2 and water, and are not being held in the water. This proves that the use of Oil Spill Eater II would not affect the ground water where treatment takes place.

This was a tough test for OSEII using older contaminated soil and sterile water, but it proves substantially that OSEII is a very viable bioremediation product to reduce the TPH from the soil.

This test was stopped short of obtaining the State of Alaska's 100 ppm reduction level. The reason the test was stopped short was because the cleanup company wanted to verify that biodegradation did occur and they felt that once it starts, only some outside action could stop bioremediation. The cleanup company was in a hurry to get their cleanup started.

Steven Pedigo
Chairman

NORTHERN TESTING LABORATORIES, INC.

2505 FAIRBANKS STREET
600 UNIVERSITY PLAZA WEST, SUITE A

ANCHORAGE, ALASKA 99503
FAIRBANKS, ALASKA 99709

907-277-6378 • FAX 274-0545
907-479-3115 • FAX 479-0547

March 21,1990

Mr. Steve Karcz

P.O. Box 190151
Anchorage, AK 99519

Dear Mr. Karcz:

This letter is to report the results of the test that was performed by Northern Testing Laboratories, Inc. on Sky Blue Chems Oil Eater II.

A sample of soil contaminated with petroleum hydrocarbons was provided to Northern Testing on March 7, 1990. A pretreatment EPA 418.1 determination was done in duplicate, using approximately 15 grams of soil per sample. Pretreatment levels were found to be 737 and 603 mg/dry kg.

The measurement for the soils were conducted by volume since the treatment instructions are based on soil volume. Five hundred milliliters of soil were divided into two portions of 250 ml each, for treatment and control. Each portion was spread evenly in the bottom of a clean, freon-rinsed 2000-ml beaker, forming a layer approximately two centimeters deep. Seven milliliters of product were diluted to 700 ml with deionized water.

The treatment consisted of three applications (approximately 233 ml each) of diluted product at approximately 48-hour intervals. The liquid was simply poured over the top of the soil layer. At the time of each application, the control portion received 233 ml of deionized water. Between treatments, the beakers were covered tightly with aluminum foil and held at approximately 62 degrees Fahrenheit.

Treatment 1: Friday March 9 at 5:00 p.m.
2: Sunday March 11 at 2:00 p.m.
3: Tuesday March 13 at 4:45 p.m.

The treatment ended Thursday March 15 at 7:00 p.m. when the treated and control soils were drained. On Friday March 16, samples of approximately 15 grams were taken from each portion and analyzed for TPH, yielding the following results:

Control sample: 767 mg/dry kg
Treated sample: 391 mg/dry kg

Sincerely,

Eileen Herring

Eileen Herring
Chemist

NORTHERN TESTING LABORATORIES, INC.

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ANCHORAGE, ALASKA 99503
FAIRBANKS, ALASKA 99709

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907-479-3115 • FAX 479-0547

Quality Control Report

Client: Sky Blue Chems.
ID#: A030790-4

Listed below are quality control assurance reference samples with a known concentration prior to analysis. The acceptable limits represent a 95% confidence interval established by the Environmental Protection Agency or by our laboratory through repetitive analyses of the reference sample. The reference samples indicated below were analyzed at the same time as your sample, ensuring the accuracy of your results.

Sample #	Parameter	Unit	Result	Acceptable Limit
EPA 379-1	Oil & Grease	mg/l	19.1	16.6 - 23.4

Reported By: Date: 03/20/90

Francois Rodigari, Anchorage Operations Manager

NORTHERN TESTING LABORATORIES, INC.

2505 FAIRBANKS STREET
600 UNIVERSITY PLAZA WEST, SUITE A

ANCHORAGE, ALASKA 99503
FAIRBANKS, ALASKA 99709

907-277-8378 • FAX 274-9645
907-479-3115 • FAX 479-0547

P.O. Box 190151
Anchorage, AK. 99519

Attn: Steve Karcz

Date Arrived: 03/07/90
Time Arrived: -
Date Sampled: 03/02/90
Time Sampled: 1151
Date Completed: 03/19/90

Source: Firestone-Northern Lights
Sample ID#: A030790-4

NTL ID#	Client ID	mg/dry kg	% Solids %
Total Petroleum Hydrocarbons: EPA Method 418.1			
A030790-4	Pre-treatment	737/603	75.9
	Treated	391	75.8
	Control	767	79.0

Reported By: Date: 03/20/90

NORTHERN TESTING LABORATORIES, INC.

2505 FAIRBANKS STREET
600 UNIVERSITY PLAZA WEST, SUITE A

ANCHORAGE, ALASKA 99503
FAIRBANKS, ALASKA 99709

907-277-8378 • FAX 274-9645
907-479-3115 • FAX 479-0547

Date Arrived: 03/07/90
Time Arrived: -
Date Sampled: 03/02/90
Time Sampled: 1151
Date Completed: 03/23/90

Attention: Steven Pedigro

Source: Supernate of treated soil
Sample ID#: A030790-4

NTL ID#	Client ID	mg/l
Total Petroleum Hydrocarbons: EPA Method 418.1		
A030790-4	Supernate of treated soil from test of Oil Spill Eater II conducted 03/09/90 - 03/19/90	2.0

Reported By: Date: 03/23/90

Francois Rodigari, Anchorage Operations Manager

NORTHERN TESTING LABORATORIES, INC.

2606 FAIRBANKS STREET
600 UNIVERSITY PLAZA WEST, SUITE A

ANCHORAGE, ALASKA 99503
FAIRBANKS, ALASKA 99709

907-277-6578 • FAX 274-9644
907-479-3115 • FAX 479-0547

Quality Control Report
==========

Client: Sky Blue Chem
ID#: A030790-4

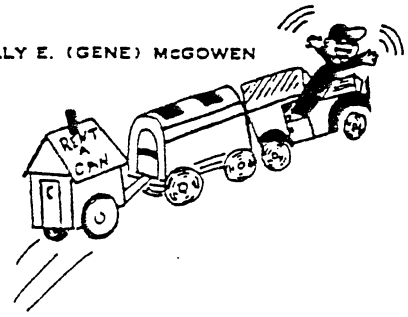
Listed below are quality control assurance reference samples with a known concentration prior to analysis. The acceptable limits represent a 95% confidence interval established by the Environmental Protection Agency or by our laboratory through repetitive analyses of the reference sample. The reference samples indicated below were analyzed at the same time as your sample, ensuring the accuracy of your results.

Sample #	Parameter	Unit	Result	Acceptable Limit
EPA WP379-1	Oil and Grease	mg/l	19.7	16.6 - 23.5

Reported By: Date: 03/23/90

Francois Rodigari, Anchorage Operations Manager

BILLY E. (GENE) McGOWEN



Rent-A-Can Toilet Co.

POST OFFICE BOX 433
EAGLE RIVER, ALASKA 99577

PHONE 694-9202
ANS. SER. 272-3722

STATE OF ALASKA
DEPARTMENT OF ENVIRONMENTAL CONSERVATION
ANCHORAGE DISTRICT OFFICE
800 E. DIAMOND BLVD. SUITE 3-470
ANCHORAGE, ALASKA 99515

ATTN: ROBERT WEIMER, ENVIRONMENTAL SPECIALIST

RE: SPILL # 91-2-1-1-295-1 FILE # L55138

ENCLOSED FOR YOU IS A REPORT PERTAINING TO THE CLEANUP OF THE CONTAMINATED SOIL INVOLVED WITH THE ABOVE SITE SPILL #. WE WANTED TO SEE IF THE COMMERCIAL PRODUCT OIL SPILL EATER, MANUFACTURED BY OSEI CORPORATION, WOULD MITIGATE THE HYDROCARBON CONTAMINATION. BECAUSE OF THE SMALL QUANTITY OF CONTAMINATION, WE TREATED ALL THE SOIL AT THE SAME TIME INSTEAD OF JUST A SMALL TRIAL PLOT. AS YOU CAN SEE IN THE REPORT, THE SOIL HAS BEEN CLEANED TO BELOW THE ADEC GUIDELINE OF 100 PPM. THEREFORE, WE REQUEST THAT YOU ACCEPT THIS REPORT AS A REMEDIATION PLAN AND ALSO GRANT US A SITE CLOSURE PER THE RESULTS OF THE INCLUDED FINAL TEST RESULT OF THE BIOREMEDIATION. WE ALSO ARE REQUESTING TO SPREAD THE CLEAN SOIL ON OUR PARKING LOT AS ADDITIONAL AERATION WILL CONTINUE THE BIORMEDIATION PROCESS TO UNDETECTABLE LIMITS ASSUMING THERE IS NO RUN OFF FROM SPRINGBROOK DRIVE WHICH WE FRONT ON AND IS OILED REGULARLY. WE WILL NOT SPREAD THE SOIL UNTIL AFTER YOUR REVIEW AND APPROVAL.

REGARDS,

WAYNE CROMWELL
MANAGER

WC/CS

OIL SPILL EATER INTERNATIONAL, CORP.

Corporate Office: 10/96
OSEI, CORP.
13127 Chandler Drive
Dallas, TX 75243
(972) 669-3390
(972) 644-8359 (FAX)

P. O. Box 190151
Anchorage, Alaska
99519
907/248-9955
907/248-2604 (fax)
(Obsolete
address/phone)

RENT A CAN

EAGLE RIVER, ALASKA

BIOREMEDIATION OF CONTAMINATED SOILS

AUGUST 28, 1992

For: Mr. Wayne Cromwell
Rent a Can
P.O. Box 770433
Eagle River, Alaska
99577

Prepared by: Steve Karcz
OSEI of Alaska
PO Box 190151
Anchorage, Alaska
99519

This report represents the results of the bioremediation of contaminated soil located at the Rent a Can Shop, 12211 Springbrook Drive, Eagle River, Alaska. The goal was to reach a total petroleum hydrocarbon (TPH) level acceptable to the Alaska Department of Conservation (ADEC) by bioremediation using Oil Spill Eater (OSE), a biocatalytic nutrient, to enhance the growth of the local indigenous bacteria found in the contaminated soil. The acceptable level to be low enough to dispose of the soil as back fill on site. This report shows that independent companies with petroleum contaminated soils can clean the soil very inexpensively on site.

THE NUTRIENT

Oil Spill Eater is a biodegradable, non-toxic, water soluble, liquid nutrient. Oil Spill Eater stimulates and accelerates natural biological reactions. There are no petroleum components or any cultured bacteria in OSE. Oil Spill Eater rapidly grows the existing hydrocarbon degrading bacteria into large colonies quicker than fertilizers due to it's nutrient components. Oil Spill Eater's use in Alaska and abroad has been proven as a most effective means of mitigating hydrocarbons.

CONTAMINATED SOIL

The contamination was believed to be diesel fuel which leaked from an underground storage tank. The soil was removed and stockpiled outside on a poly membrane. The amount was estimated to be 1 1/2 cubic yards. The TPH test showed a level of 3060 ppm by EPA test method 8100 MOD. Other EPA test methods, 8015 and 8020, also indicated diesel fuel was the probable contamination. The soil was permeable, consisting of mostly gravel up to two inches mixed with organics.

REMEDIATION PLAN

A remediation plan was designed allowing the clean up to be conducted in treatment cells on location inside the Rent A Can shop. The amount of contaminated soil was relatively small allowing for remediation of all the material in a controlled environment. This also allowed easy and convenient access by their employees for the daily remediation labor.

TREATMENT CELL

The treatment cells consisted of 55 gallon drums without lids. A six inch tall wooden grate was constructed to create a void at the bottom as a recovery sump. Filter fabric was then placed on top of the grate to prevent any soil from migrating into the sump. A two inch hole was drilled in to the grate in which a piece of two inch diameter PVC pipe was placed. The pipe was one-fourth of an inch from the bottom and reached to the rim of the drum. The pipe served as both a monitoring tube and as recovery access. A hand pump could then be placed into the PVC pipe and the effluent water/nutrient pumped back up so to percolate back down through the soil. The smallest aquarium air pump that could be found was obtained and air was injected through rubber tubing into the water in the sump. The drums were placed in the middle of the shop so to prevent any foreign contamination from entering the cells.

WORK PLAN

The work plan consisted of placing the contaminated soil into the drum, treating it with Oil Spill Eater, and recycle the nutrient water mixture through the soil daily. The contaminated soil was mixed in a small loader prior to placement in the drum. This was done to help get a consistent TPH level throughout the drums of soil. Previous projects have shown that consistency of contamination throughout a soil pile is not naturally congruent due to excessive excavation of cleaner soils. Approximately 5.6 cubic feet fit into each created treatment cell.

The amount of Oil Spill Eater used was determined by the manufacturers formula. The treatment for light petroleums required .22 gallons of Oil Spill Eater per cubic yard of soil. The amount of OSE required 5.8 ounces for 5.6 cubic feet of soil. This amount was then mixed at a 1 to 100 ratio with unchlorinated water. A well located on site was the water source for this project. Five hundred eighty ounces of water was mixed with the 5.8 ounces of OSE per drum.

The nutrient mixture was applied with a sprinkler type watering can at the surface of the drum. The rate of application was slow enough to prevent ponding on the surface to ensure a consistent percolation through the soil matrix.

The nutrient solution was pumped to the surface daily so it could percolate back down through the soil. This kept the soil at a high moisture content. The treatment began on April 9, 1992, and the pumping schedule continued through July 10, 1992 on a regular basis. There was no pumping on the weekends. Plain, unchlorinated water was added as needed to maintain moisture content.

SAMPLING

An initial sample was taken prior to the nutrient application to determine a starting TPH. Twelve weeks later, the first treated sample was extracted after the initial treatment. The sample was taken approximately 10 inches down into the matrix with the exact location marked. This would provide that both the initial and treated samples came from the exact location which would lessen any contamination inconsistency throughout the soil. The ten inch depth was chosen to assure volatilization or evaporation would not be a factor on the lighter end hydrocarbons. The samples were then taken to Chemical & Geological Laboratory in Anchorage for testing. The initial sample was tested by EPA Method 8100 Modified. This test was chosen as previous tests were available using the 8100 modified. The after treatment test was done by EPA Method 418.1.

RESULTS

The initial TPH value after the soil had been homogenized in the back hoe bucket was 572 ppm. Test results prior to the bioremediation project showed 3060 ppm with the sample being taken from the known "hot" spot. The homogenizing was necessary for this project to show a consistent level of contamination throughout the soil so the testing would represent the entire drums of soil. Due to OSE's many test results, the first treated sample was not extracted for twelve weeks. This time frame was chosen as previous tests have shown the biodegradation curve and testing would not be economic. The first treated sample by Method 418.1 had a value of 76.6 ppm. With this value being below the Alaska Department of Environmental Conservation's acceptable value of 100 ppm as being clean, the tests were terminated.

CONCLUSION

After 12 weeks of bioremediation, the homogenized soil which had a consistent contamination of 572 ppm (8100 Modified) was reduced to 76.6 ppm (418.1).

With this final value achieved below ADEC's acceptable guideline limits, I submit this report to you and recommend you contact ADEC for their approval to spread the cleaned soil on your lot. This report should suffice as both a Remediation Plan and a Closure Request.

STATE OF ALASKA

WALTER J. HICKEL, GOVERNOR

DEPT. OF ENVIRONMENTAL CONSERVATION

ANCHORAGE DISTRICT OFFICE
800 E. DIMOND BLVD., SUITE 3-470
ANCHORAGE, ALASKA 99515

(907) 349-7755

December 7, 1992

Wayne Cromwell
Rent-A-Can Toilet Co.
P.O. Box 433
Anchorage, Ak 99577

Subject: 12211 Springbrook Drive, Eagle River site - Soil Disposal
File#: L55.138

Dear Mr. Cromwell:

The Department has completed the review of the information you submitted and the file regarding the 1.5 cubic yards of remediated soils in drums at the above referenced site. The soils have been remediated to the most stringent cleanup levels and are approved to be spread on-site (as per verbal approval to you on 9/21/92).

If you have any questions concerning this letter, please contact me at the Anchorage District Office at 349-7755.

Sincerely,

Robert Weimer

Robert Weimer
Environmental Specialist

RW/cf

OIL SPILL EATER INTERNATIONAL, CORP.

13127 Chandler Drive
Dallas, Texas 75243
(972) 669-3390
(972) 644-8359 Fax
admirallively@msn.com

In-Situ Underground Cleanup of Heating Oil

In November, 1996 Alpha Geoscience used **OSE II** to perform an "*In-Situ Cleanup*" of 115 cubic yards of kerosine contaminated soil in a trailer park in Highland Falls, New York. The in-situ cleanup was necessary since removing the contaminated soil could not be done without moving buildings and porches.

Attached is Alpha Geoscience's December 1, 1997 letter to the New York State Department of Environmental Control (NYSDEC) reporting their final data collected and requesting a "*closure letter*" from the state of New York.

The NYSDEC report form and "*closure letter*" follows the Geoscience's report and their request for a closure letter. Note that the NYSDEC states "***Cleanup Complete.* NFA (No Further Action).**"

This cleanup proves how effective **OSE II** is for "*in-situ cleanups*" under buildings and concrete or asphalt areas.

O.A. (George) Lively
Rear Admiral (RET)
President

OAL/eem

Enclosure

ALPHA
GEOSCIENCE

Geology
Hydrology
Remediation
Water Supply

December 1, 1997

Mr. David Traver
NYSDEC Region 3
21 South Putt Corners Road
New Paltz, New York 12561-1696

Re: Summary and Results of In Situ Soil Remediation
42 Hudson View Terrace, Highland Falls, New York
NYSDEC Spill No. 95-16786

Dear Mr. Traver:

This letter report summarizes the remediation efforts performed on November 7 and 8, 1996, July 24 and November 3, 1997, at the above referenced site. The work was performed in accordance with the Revised Remedial Work Plan, prepared by Alpha Geoscience, dated September 25, 1996, and approved by the New York State Department of Environmental Conservation (NYSDEC) on October 21, 1996. A description of the work performed is presented below.

November, 1996 Activities

A pre-remediation composite soil sample (SS-1) was collected for laboratory analysis. The sample was collected to provide a baseline for comparison with post-remediation sampling to determine the effectiveness of remediation. Composite sample SS-1 was collected from a depth of 0.5 to 2.5 feet at four locations identified as being within the contaminated area during the previous spill investigation. The approximate sampling locations are shown on the attached Figure 1. The composite sample was retained in laboratory-supplied containers, placed on ice in a chilled cooler, and delivered to a NYS Department of Health (DOH)-certified laboratory following chain of custody protocol. The sample was analyzed for volatile organic compounds (VOCs) via EPA Method 8021, and semi-VOCs via EPA Method 8270, in accordance with the NYSDEC Petroleum-Contaminated Soil Guidance Policy, STARS Memo #1. A copy of the laboratory analytical report is provided in Attachment No. 1. The results indicate elevated levels of VOCs in the soil, totaling approximately 35 parts per million (ppm). No semi-VOCs were detected; however, the detection limit was elevated (2.0 ppm) because of the high VOC concentrations, possibly masking the presence of semi-VOCs.

An earth berm was constructed to prevent surface water runoff to the small stream during application of the bioenhancement product. The berm was constructed between trailers number 42 and 43 on the east side of the stream (Figure 1). The berm is approximately 10 inches high and is covered and secured with plastic sheeting to prevent erosion of, or infiltration through, the berm.

After construction of the berm, the soil was prepared by rototilling the accessible affected area. Remedial efforts were implemented by applying a NYSDOH-approved bioenhancement product known as Oil Spill Eater II (OSE II). Application of the OSE II was performed by mixing the soluble blend with water and spraying the mixture onto the entire area to be treated. The OSE II was applied at a concentration of approximately 0.38 gallons per cubic yard of soil. Approximately 19 gallons of OSE II was applied each day for two consecutive days. The soil was tilled again following application on the first day. Saturated surface soil conditions precluded tilling following the OSE II application on the

400 Trillium Lane • Albany, New York 12203 • (518) 452-0096 • FAX (518) 452-7820

second day. The OSE II was applied according to the manufacturers specifications for application. The accessible areas were covered and secured with black plastic sheeting to facilitate bacterial growth by warming the soil via solar convection.

July, 1997 Activities

The plastic sheeting was removed and a landscaping company installed a new grass sod lawn in the area of the OSE II application during June, 1997. Alpha personnel revisited the site on July 24, 1997 to screen the subsurface soil and monitor the progress of remediation. A soil sample was collected at or very near each of the four sampling locations shown on Figure 1. The soil samples were screened via headspace analysis utilizing an HNU DL-101 photoionization detector (PID) calibrated with isobutylene gas. The results of PID screening indicated significantly reduced measurements compared to PID screening during the initial site characterization performed in May, 1996, indicating the bioremediation was working effectively. A copy of the organic vapor screening log is provided in Attachment No. 2.

A composite soil sample, designated Comp-1, was collected from the four sampling locations using the same sampling procedures used in November, 1996. The sample was analyzed for total petroleum hydrocarbons (TPH) to provide a relative indication of the amount of contamination remaining. The results of the analysis indicated a TPH concentration of 88 milligrams per kilogram (mg/kg). A copy of the laboratory analytical report is provided in Attachment No. 3.

November, 1997 Activities

On November 3, 1997, Alpha personnel collected soil samples, designated CS-1, CS-2, CS-3 and CS-4, at or near each of the four locations shown on Figure 1. A surface water sample, designated W-1, and a sediment sample, designated SW-1, were collected from the nearby small stream. Headspace screening of the soil, water and sediment samples was performed utilizing the PID. Screening of samples W-1, SW-1, CS-1 and CS-4 via the PID indicated measurements at the instrument background level. Soil samples CS-2 and CS-3 measured 3.8 parts per million (ppm) and 14 ppm above instrument background, respectively. A copy of the organic vapor screening log is provided in Attachment No. 2.

A composite soil sample, designated CS-1, was collected from the four locations using the methods described above. The sample was analyzed for STARS VOCs via TCLP EPA Method 8021 and STARS semi-VOCs via TCLP EPA Method 8270, in accordance with the approved work plan. The results of analysis indicated no semi-VOC compounds were detected. The results of analysis for VOCs indicated four compounds were detected in concentrations slightly exceeding their respective NYSDEC STARS guidance values. The compounds are 1,3,5-trimethylbenzene detected at 8 parts per billion (ppb), tert-butylbenzene (coelutes with 1,2,4-trimethylbenzene) at a total of 16 ppb, and naphthalene at 18 ppb. A copy of the laboratory analytical report is attached.

Summary and Recommendation

A summary of the site remediation and recommendations is presented below.

- The petroleum-impacted soil area was treated in accordance with the NYSDEC-approved Work Plan and the manufacturers recommendations utilizing a bioremedial enhancement product called OSE II.

- The results of PID screening of individual soil sample locations indicates a significant reduction of petroleum compounds since remedial efforts were initiated in November, 1996.

- The results of PID screening of water and sediment collected from the nearby small stream indicate contamination has not migrated to the stream.

- The analytical results of post-remedial soil samples indicates the OSE II has effectively stimulated activity of indigenous bacteria resulting in substantial breakdown and reduction of petroleum compounds. The soil generally meets NYSDEC soil cleanup guidance criteria specified in STARS Memo #1. Continued bacterial activity will further reduce and breakdown petroleum compounds.

No further investigation or remediation is necessary for this site, based on the investigation and analytical data. We hereby request the NYSDEC close the spill file for this site.

If you have any questions regarding this report, please contact me or Tom Johnson.

Sincerely,
Alpha Geoscience

Michael S. Ralbovsky
Hydrogeologist

MSR:ce
attachment
cc: Mr. Don Abel (La Marche)
d:/.../highland/closure.ltr

NYSDEC SPILL REPORT FORM

DEC REGION# 3 (New Paltz) SPILL NUMBER 9516786
SPILL NAME: SAMADI RESIDENCE DEC LEAD: MCCARTHY
CALLER'S NAME: VICTROIA SAMADI NOTIFIER'S NAME: VICTROTIA SAMADI
CALLER'S AGENCY: CITIZEN NOTIFIER'S AGENCY: CITIZEN
CALLER'S PHONE: (914) 446-0519 EXT. NOTIFIER'S PHONE: (914) 446-0519 EXT.

SPILL DATE: 03/28/96 TIME: 12:00
CALL RECEIVED DATE: 03/28/96 TIME: 15:38 RECEIVED BY CID #: 257

	Material Spilled	Mat. Class	Am't Spilled	Units	Am't Recovered
1)	KEROSENE	(Pet)-Haz-Other-Unk.	Unknown	(Gal) - Lbs	Unknown
2)		Pet-Haz-Other-Unk.		Gal - Lbs	
3)		Pet-Haz-Other-Unk.		Gal - Lbs	
4)		Pet-Haz-Other-Unk.		Gal - Lbs	

SPILL LOCATION

PLACE: SAMADI RESIDENCE
STREET: 42 HUSDONVIEW TERR
T/C/V: HIGHLAND FALLS CO: ORANGE
CONTACT: VICTROIA SAMADI
PHONE: (914) 446-0519 EXT.

POTENTIAL SPILLER

NAME: SAMADI RESIDENCE
STREET: 42 HUSDONVIEW TERR
CITY: HIGHLAND FALLS
STATE: NY ZIP:
CONTACT: VICTROIA SAMADI
PHONE: (914) 446-0519 EXT.

SPILL CAUSE

Human Error	Tank Test Failure*	(Tank Failure)
Traffic Accident	Housekeeping	Tank Overfill
Equipment Failure	Deliberate	Other
Vandalism	Abandoned Drums	Unknown

SPILL SOURCE

Gas Station	(Private Dwelling)	Non-Maj Facility
Passenger Vehicle	Vessel	Comm/Indust
Comm. Vehicle	Railroad Car	Non-Comm/Instit
Tank Truck	Major Facility	Unknown

RESOURCE AFFECTED

(On Land)	Groundwater	Air
In Sewer	Surface Water**	

**WATERBODY:

SPILL REPORTED BY

(Responsible Party)	Tank Tester	Local Agency
Affected Persons	DEC	Federal Gov't
Police Department	Citizen	Other
Fire Department	Health Dept.	

CALLER REMARKS: tank leaked unknown if company did cleanup when they replaced tank

*PBS Number	Tank Number	Tank Size	Test Method	Leak Rate

PRIMARY CONTACT CALLED DATE: TIME: hrs. REACHED DATE: TIME: hrs.
SECONDARY CONT. CALLED DATE: TIME: hrs. FAXED BY CID#:

PIN #	T & A	Cost Center	ISR to Central Office
Cleanup Ceased	Meets St'ds YES	Last Inspection	Penalty NO
RP-CUI	ENF-INIT	INVES-COM	CAP
UST Trust Eligible NO	Site: A B (C) D E	Resp. Party 1 2 3 4 5 (6)	Reg Close Date 12/19/97

Created on 03/28/96 Last Updated on 01/06/98 Is Updated? NO EDO DATA INPUT [ ]
Date Printed: 03/04/98

PrintFor 10/9/98 AAA

DEC REMARKS

12/19/97 RECEIVED REPORT OF REMEDIAL ACTION FROM ALPHA GEOSCIENCE. CLEANUP COMPLETE. NFA;

13127 Chandler Drive
Dallas, Texas 75243
(972) 669-3390
(972) 644-8359 (Fax)
admirallively@msn.com

SUMMARY

BIOREMEDIATION OF CHLORINATED HYDROCARBONS

Key Bank in Anchorage, Alaska was in possession of a contaminated property which contained dichloralbenze, low levels of BETX and a total TPH of 7,500. This test verified what had previously been hypothesized about OSEII. OSEI Corporation had predicted OSEII would bioremediate chlorinated hydrocarbons, as well as PCBS and various other toxic wastes.

The chlorinated hydrocarbons were reduced to undetectable levels and the TPH was reduced from 7,500 to 1,890 in less than 30 days. However, the lab ran out of test material, but the testing performed definitely shows the extent to which OSEII can biodegrade chlorinated hydrocarbons.

Steven Pedigo
Chairman

NORTHERN TESTING LABORATORIES, INC.

3330 INDUSTRIAL WAY
2505 FAIRBANKS STREET
FAIRBANKS, ALASKA 99701
ANCHORAGE, ALASKA 99503
(907) 456-3116 • FAX 456-3125
(907) 277-8378 • FAX 274-9645

February 12, 1991

Mr. Steve Karcz
P.O. Box 190151
Anchorage, AK 99519

Dear Mr. Karcz:

This letter is to report the results of the test that was performed by Northern Testing Laboratories, Inc. on Sky Blue Chems Oil Eater II.

A sample of contaminated soil was provided to NTL on January 3, 1991. 100 grams of soil was measured to be approximately 62.5 mls in volume. On January 4 the soil was spiked with a 1ppm, 1,2 Dichlorobenzene spike. A dilution of 1:100 OSE II was made with the provided well water and OSE concentrate. This solution was then applied to the soil sample at a rate of 22 gallons per yard of soil and mixed well with the soil sample. A sample of soil was then extracted for an EPA 418.1 (TPH) and an EPA 8020 and a percent solid.

The 8020 was analyzed on day one and the 418.1 on day three. Because the 418.1 was not analyzed on day one, a second portion of the original provided sample (without a spike) was extracted and treated with the OSE II at above rate and analyzed for 418.1 only to provide an approximated day one baseline to compare with the next seventh day reading .

The sample was then covered with foil and held with a rubber band and left at room temperature (approx. 20 deg C.) to incubate.

The moisture content was visually determined and an amount of well water added to the soil at seven day intervals. The amount of well water to be added was determined by visually estimating the moisture content of the soil; water was added to increase the visible moisture but without leaving standing water.

Additional portions of the sample were extracted for 8020's and 418.1's on the following days: January 11:Day 8, January 18:Day 15, January 25:Day 22 and February 4:Day 32. For the second sample used to determine the 418.1 baseline a second portion of the sample was extracted and analyzed on January 11:Day 7.

SKYBLUE.DOC

NORTHERN TESTING LABORATORIES, INC.

3330 INDUSTRIAL WAY
2505 FAIRBANKS STREET
FAIRBANKS, ALASKA 99701
ANCHORAGE ALASKA 99503
(907) 456-3116 • FAX 456-3125
(907) 277-8378 • FAX 274-9645

February 12, 1991

The following results were found after analysis:

Day	NTL ID#	418.1ppm	8020ppm 1,2 DCB	1,3 DCB	1,4 DCB	Xyl.	Tol.
1	A107826	----	0.7	0.3	0.2	2.5	<0.1
3	A107826	7500	----	----	----	----	----
8	A107829	7310	2.1	<.02	<.1	2.2	<.05
15	A108082	6320	----	----	----	----	----
15	A108348	----	<0.2	<0.2	<0.2	1.3	<0.1
22	A108175	4035	<0.2	<0.2	<0.2	1.5	<0.1
32	A108299	1890	----	----	----	----	----

(For second 418.1 baseline sample)

1	A108042	11900	----
8	A108054	11600	----

The elevated level of 1,2 DCB on Day 8 was apparently due to an inability to homogeneously mix the concentrated spike throughout the sample. However all the other parameters evidenced a steady decrease in concentration.

The experiment procedure was terminated because of lack of remaining spike-treated soil. If further soil had been available the procedure would have continued until no amount of parameters for the TPH or 8020 remained.

Sincerely,

Donna Sherwood

Donna Sherwood
Environmental Analyst

SKYBLUE.DOC

NORTHERN TESTING LABORATORIES, INC.

3330 INDUSTRIAL WAY, FAIRBANKS, ALASKA 99701 (907) 456-3116 • FAX 456-3125
2505 FAIRBANKS STREET, ANCHORAGE, ALASKA 99503 (907) 277-8378 • FAX 274-9645

Sky Blue Chems
P.O. Box 190151
Anchorage AK 99519

Attn: Steve Karcz

Report Date: 01/23/91

Date Arrived: 01/03/91
Date Sampled: 01/03/91
Time Sampled: -
Collected By: SK

Flag Definitions
U = Below Detection Limit
DL Stated in Result
B = Below Regulatory Min.
H = Above Regulatory Max.
E = Below Detection Limit
Estimated Value

Our Lab #: A107826
Location/Project: -
Your Sample ID: Rogers & Babler (before)1
Sample Matrix: Soil
Comments: Revised Transmittal.

Method	Parameter	Units	Result	Flag	Date Analyzed
EPA 160.3	Solids	%	86.9		01/14/91
EPA 418.1	Total Petroleum Hydrocarbons	mg/dry kg	7500		01/07/91
EPA 8020	Benzene	mg/dry kg	0.10	U	01/11/91
	Chlorobenzene	mg/dry kg	0.10	U	
	1,2-Dichlorobenzene	mg/dry kg	0.70		
	1,3-Dichlorobenzene	mg/dry kg	0.30		
	1,4-Dichlorobenzene	mg/dry kg	0.20	U	
	Ethylbenzene	mg/dry kg	0.10	U	
	Toluene	mg/dry kg	0.10	U	
	Xylenes	mg/dry kg	2.50		

William E. Buchan

Reported By: William E. Buchan
Anchorage Operations Manager

NORTHERN TESTING LABORATORIES, INC.

3330 INDUSTRIAL WAY, FAIRBANKS, ALASKA 99701 · (907) 456-3116 · FAX 456-3125
2505 FAIRBANKS STREET, ANCHORAGE, ALASKA 99503 · (907) 277-8378 · FAX 274-9645

Sky Blue Chems
P.O. Box 190151
Anchorage AK 99519

Attn: Steve Karcz

Report Date: 01/23/91

Date Arrived: 01/03/91
Date Sampled: 01/03/91
Time Sampled: -
Collected By: SK

Flag Definitions
U = Below Detection Limit
DL Stated in Result
B = Below Regulatory Min.
H = Above Regulatory Max.
E = Below Detection Limit
Estimated Value

Our Lab #: A107829
Location/Project: -
Your Sample ID: Rogers & Babler (after)
Sample Matrix: Soil
Comments: Revised Transmittal.

Method	Parameter	Units	Result	Flag	Date Analyzed
EPA 160.3	Solids	%	86.9		01/21/91
EPA 418.1	Total Petroleum Hydrocarbons	mg/dry kg	7310		01/12/91
EPA 8020	Benzene	mg/dry kg	0.05	U	01/18/91
	Chlorobenzene	mg/dry kg	0.05	U	
	1,2-Dichlorobenzene	mg/dry kg	2.10		
	1,3-Dichlorobenzene	mg/dry kg	0.02	U	
	1,4-Dichlorobenzene	mg/dry kg	0.10	U	
	Ethylbenzene	mg/dry kg	0.05	U	
	Toluene	mg/dry kg	0.05	U	
	Xylenes	mg/dry kg	2.20		

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3330 INDUSTRIAL WAY
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FAIRBANKS, ALASKA 99701
ANCHORAGE ALASKA 99503
(907) 456-3116 • FAX 456-3125
(907) 277-8378 • FAX 274-9645

Sky Blue Chems.

Attn: Steve Karcz

Report Date: 02/08/91

Date Arrived: 02/07/91
Date Sampled: 01/18/91
Time Sampled: -
Collected By: DS

Flag Definitions
U = Below Detection Limit
DL Stated in Result
B = Below Regulatory Min.
H = Above Regulatory Max.
E = Below Detection Limit
Estimated Value

Our Lab #: A108348
Location/Project: -
Your Sample ID: 14 Days/A108082
Sample Matrix: Soil
Comments:

Method	Parameter	Units	Result	Flag	Date Analyzed
EPA 160.3	Solids	%	86.9		01/18/91
EPA 8020	Benzene	mg/dry kg	0.10	U	02/07/91
	Chlorobenzene	mg/dry kg	0.10	U	
	1,2-Dichlorobenzene	mg/dry kg	0.20	U	
	1,3-Dichlorobenzene	mg/dry kg	0.20	U	
	1,4-Dichlorobenzene	mg/dry kg	0.20	U	
	Ethylbenzene	mg/dry kg	0.10	U	
	Toluene	mg/dry kg	0.10	U	
	Xylenes	mg/dry kg	1.30		

William E. Buchan

Reported By: William E. Buchan
Anchorage Operations Manager

NORTHERN TESTING LABORATORIES, INC.

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2505 FAIRBANKS STREET, ANCHORAGE ALASKA 99503 (907) 277-8378 • FAX 274-9645

Sky Blue Chem
13355 Noel Road, 5th Floor
1 Galleria Tower 503-5999
Dallas TX 75240

Attn: -

Report Date: 02/01/91

Date Arrived: 01/18/91
Date Sampled: 01/18/91
Time Sampled: -
Collected By: DS

Flag Definitions
U = Below Detection Limit
DL Stated in Result
B = Below Regulatory Min.
H = Above Regulatory Max.
E = Below Detection Limit
Estimated Value

Our Lab #: A108082
Location/Project: -
Your Sample ID: 14 days
Sample Matrix: Soil
Comments:

Method	Parameter	Units	Result	Flag	Date Analyzed
EPA 160.3	Solids	%	86.9		01/18/91
EPA 418.1	Total Petroleum Hydrocarbons	mg/dry kg	6320		01/18/91

William E. Buchan

Reported By: William E. Buchan
Anchorage Operations Manager

NORTHERN TESTING LABORATORIES, INC.

3330 INDUSTRIAL WAY
2505 FAIRBANKS STREET

FAIRBANKS, ALASKA 99701
ANCHORAGE, ALASKA 99503

(907) 456-3116 • FAX 456-3125
(907) 277-8378 • FAX 274-9645

Sky Blue Chems
13355 Noel Road, 5th Floor
1 Galleria Tower 503-5999
Dallas TX 75240

Attn: Steve Karcz

Report Date: 02/12/91

Date Arrived: 01/25/91
Date Sampled: 01/25/91
Time Sampled: -
Collected By: SK

Flag Definitions
U = Below Detection Limit
DL Stated in Result
B = Below Regulatory Min.
H = Above Regulatory Max.
E = Below Detection Limit
Estimated Value

Our Lab #: A108175
Location/Project: -
Your Sample ID: 28 Days 4th Sample
Sample Matrix: Soil
Comments:

Method	Parameter	Units	Result	Flag	Date Analyzed
EPA 160.3	Solids	%	86.9		01/25/91
EPA 418.1	Total Petroleum Hydrocarbons	mg/dry kg	4035		01/25/91
EPA 8020	Benzene	mg/dry kg	0.10	U	02/08/91
	Chlorobenzene	mg/dry kg	0.10	U	
	1,2-Dichlorobenzene	mg/dry kg	0.20	U	
	1,3-Dichlorobenzene	mg/dry kg	0.20	U	
	1,4-Dichlorobenzene	mg/dry kg	0.20	U	
	Ethylbenzene	mg/dry kg	0.10	U	
	Toluene	mg/dry kg	0.10	U	
	Xylenes	mg/dry kg	1.50		

William E. Buchan

Reported By: William E. Buchan
Anchorage Operations Manager

NORTHERN TESTING LABORATORIES, INC.

3330 INDUSTRIAL WAY, FAIRBANKS, ALASKA 99701 (907) 456-3116 • FAX 456-3125
2505 FAIRBANKS STREET, ANCHORAGE ALASKA 99503 (907) 277-8378 • FAX 274-9645

Sky Blue Chems.

Attn: Steve Karcz

Report Date: 02/06/91

Date Arrived: 02/04/91
Date Sampled: 02/04/91
Time Sampled: -
Collected By: SK

Flag Definitions
U = Below Detection Limit
DL Stated in Result
B = Below Regulatory Min.
H = Above Regulatory Max.
E = Below Detection Limit
Estimated Value

Our Lab #: A108299
Location/Project: -
Your Sample ID: -
Sample Matrix: Soil
Comments:

Method	Parameter	Units	Result	Flag	Date Analyzed
EPA 160.3	Solids		86.9		02/04/91
EPA 418.1	Total Petroleum Hydrocarbons	mg/dry kg	1890		02/04/91

William E. Buchan

Reported By: William E. Buchan
Anchorage Operations Manager

DEPARTMENT OF THE AIR FORCE
AIR FORCE BASE CONVERSION AGENCY

11 Sep 96

FROM: AFBCA/OL-H
6550 White Settlement Rd.
Ft. Worth, TX 76114

TO: Mr. George Lively
Oil Spill Eater International Corp.
~~5545 Harvest Hill, #1116~~
Dallas, TX. 75230

current address as of 10/96
13127 Chandler Drive
Dallas, TX 75243

Dear Mr. Lively

This letter gives you some background information on our circumstances and reports the results of our clean up pertaining to the petroleum hydrocarbon stained concrete floor.

During the transfer of the PCB Storage Facility to the Navy, a Texas Natural Resource Conservation Commission (TNRCC) representative found a petroleum hydrocarbon stain on the concrete floor. The stain was 4 ft x 6 ft, and hidden under a larger transformer. The TNRCC representative required swipe testing be conducted to determined the levels of PCB contamination. The results of swipe test detected a high levels of PCB contamination that required mitigation before transfer.

We received bioremediation proposals of $5,000.00 and higher from local environmental companies. However, we made an in house effort to mitigate this stain to save funds. We used a denatured alcohol to mitigate the stain and then had it tested a second time. The test results came back and the PCB levels were almost three times higher at 120 ug/ft2.

A member of the Navy gave us a half gallon of your product, Oil Spill Eater II, to try. We mixed the half gallon of Oil Spill Eater II with a gallon of water. I placed the application on the stain and kept it moist for two weeks. After two weeks, we tested the stain again for a third time. The levels of PCB contamination went from 120 ug/ft2 to only **8.2 ug/ft2**. These levels are far below the limits set forth in CFR 40 Part 761.125(3)(b)(i) and in TNRCC's Chapter 335.551, Subchapter S.

We saved the tax payers over $5,000.00. **I will personally recommend your product to everyone I meet in the environmental field.** All lab reports are attached.

Sincerely,

Elliot Smith

Elliot Smith
Engineering Technician

OIL SPILL EATER INTERNATIONAL, CORP.

13127 Chandler Drive
Dallas, Texas 75243
(972) 669-3390
(972) 644-8359 Fax
admirallively@msn.com

MARINE TOXICITY TEST SUMMARY

OSEI Corporation, in its attempt to prove ***"OIL SPILL EATER II"*** is virtually non-toxic, had the following tests performed:

The **MYSIDOPSIS BAHIA (or Mysid)** is one of the more sensitive marine organisms found in the oceans. LC50's (Lethal Concentration) is the level in which there is mortality with 50% of the species being tested. The lethal concentration calculated for OSEII on the Mysid was calculated once 10% of the test species showed equilibrium problems or mortality. At 96 hours, only 10% of the test species showed equilibrium problems or mortality at a calculated level of 2100 mg/L or 2,100 parts per million. This shows OSEII to have a low toxicity level, and had a true LC50 been performed the toxicity level would have been even lower.

The **MUMMICHOG (Fundulus Heteroclitus)** a somewhat larger organism (1 to 1.5 inches long) was tested to see how toxic OSEII was to it. 5,258 mg/L was established. 5,285 parts per million shows a very little toxicity for the Mummichog when exposed to Oil Spill Eater II.

MEDIAN LETHAL CONCENTRATIONS (LG50's) were calculated on Artemia Salina. The tests were run for 48 hours. OSEII alone tested greater than 100 mg/L so the true LG50 was not determined, but OSEII toxicity was greater than the EPA's cut-off for approving a product for the National Contingency Plan. There were other interesting facts involved with this toxicity test. The test calculation was based on using our product at a stronger concentration than our instructions allow. So at our instructed use rate, the toxicity level would have been even lower, even though the test was based on 100 mg/L or greater value. No. 2 fuel oil was tested alone and showed a level of 12.6 mg/L at 48 hours and No. 2 fuel oil and OSEII together at 48 hours showed a level of 29.4 helping prove our point that once OSEII is applied, it immediately starts detoxifying hydrocarbons so bacteria can devour the hydrocarbons. (It is more beneficial to the environment to apply OSEII immediately, than to wait around for evaporation or to try to pick up the hydrocarbons mechanically.)

OSEI Corporation feels the toxicity tests run in conjunction with OSEII help prove OSEII is virtually non-toxic. The EPA established that 35 mg/L LC50 was acceptable for a particular product to be used on the Exxon Valdez spill. If you compare OSEII to this established toxicity of 35 mg/L, then OSEII is far less toxic than that.

OSEI Corporation had two (2) fresh water toxicity tests run also. Environmental Canada, the U.S. EPA's equivalent in Canada, performed a toxicity test on rainbow trout. Rainbow trout are very sensitive fresh water species. The LC50 was greater than 10,000 mg/L. This shows OSEII to have virtually no toxicity in fresh water as well as salt water.

The other 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.

Two gallons of gasoline was poured onto a low area in a commercial business parking lot, and OSEII was applied, allowed to set 3 minutes, and then washed to another low area for collection.

Approximately 1 1/2 gallons of runoff was collected and taken to the lab where a 48 hour fathead minnow survival test was initiated. The resulting LC50 test was 9,300 mg/L which shows that gasoline which has had OSEII applied to it is rendered virtually non-toxic.

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 lot allowing the site to stay within its NPDES permitted discharge levels.

Sincerely,

Steven Pedigo
Chairman

SP/eem

OIL SPILL EATER INTERNATIONAL, CORP.

13127 Chandler Drive
Dallas, Texas 75243
(972) 669-3390
(972) 644-8359 (Fax)
admirallively@msn.com

SUMMARY

EPA/NETAC TOXICITY TEST

MYSIDOPSIS BAHIA

The Environmental Protection Agency in Gulf Breeze, Florida tested OIL SPILL EATER II Concentrate. for Toxicity using a sensitive species named "Mysidopsis Bahia". This test was in conjunction with Efficacy Tests performed by the EPA and NETAC.

The LC50 for the acute (96 hr.) test was greater than 1,900 and up to 10,000 mg/L which shows OSE II to be virtually non-toxic.

The EPA allowed the use of Inipol during the Valdez Spill and Inipol's LC50 was 135 mg/L which would seem to OSEI, Corp. to be somewhat toxic considering Environment Canada's cut off is 1,000 mg/L.

A second LC50 was performed at 7 days to see if there was any problem with chronic toxicity. The LC50 was 2,500 mg/L, which once again shows OSE II to be virtually non-toxic even when the species was exposed in a closed environment for 7 days. It would be extremely difficult for a species to be exposed to OSE II for 7 days in an open system due to currents, wind and tidal actions.

This 3rd party, U.S. EPA Toxicity Test absolutely proves OSE II is virtually non-toxic.

By: Steven R. Pedigo
Chairman/OSEI, Corp.

SRP/AJL

OIL SPILL RESPONSE BIOREMEDIATION AGENTS
EVALUATION METHODS VALIDATION TESTING
DISCUSSION OF RESULTS

The following data are provided for the oil spill response bioremediation agent producer as a means to begin to assess how this bioremediation agent may behave in response to an oil spill in the environment.

The Tier II 96-hour acute toxicity test data was conducted with Mysidopsis bahia test species. Mortality was the single measure response, therefore, survival data were used to calculate the 96-hour LC_{50}. LC_{50} is the lowest concentration effecting 50% mortality of the test organism during a 96 hour exposure period. Sub-lethal and lethal responses were noted at concentrations between 1,000-10,000 mg/L (> 1,900 mg/L) following acute exposure of M. bahia to your bioremediation product.

Oil Spill Eater II was shown to cause a statistically significant reduction ($p = 0.05$) in the survival of Mysidopsis when animals were exposed during a chronic estimator test for a 7 day period. In general, 7 day exposure (2,500 mg/L) correlated well with values calculated following the 96 hour exposure (> 1,900 mg/L).

NETAC

TIER II TOXICITY DATA

TABLE 1

ACUTE TOXICITY VALUES FOR 96 HOUR LC_{50} - *MYSIDOPSIS BAHIA*

Product	Max. Test Concentration (mg/L)	96 hour LC_{50} (mg/L)	Confidence Interval (95%)
Oil Spill Eater II	10,000	1,000-10,000[a] >1,900[b]	ND

LC_{50} = Lethal concentration of product that will cause the death of 50% of the test species population within a defined exposure time.

a = LC_{50} presented as a range of test concentrations since data were from 96-hour acute range-finding test.

b = LC_{50} presented as a single, numerical value since data were from a definitive 96-hour acute toxicity test.

ND = Not Determined

TABLE 2

CHRONIC TOXICITY VALUES FOR 7 DAY LC_{50} - *MYSIDOPSIS BAHIA*

Product	Endpoints (mg/L)		Effects Measurement	7 Day LC_{50} mg/L (95% CI)
	NOEC	LOEC		
Oil Spill Eater II	1,900 1,900 633	5,700 NE 1,900	Survival Growth Fecundity	2,500 (2,225-3,313)

NOEC = No Observable Effect Concentration
LOEC = Lowest Observable Effect Concentration
CI = Confidence Interval
NE = No Effect
Fecundity = Egg Production

As we indicated prior and to better understand the data presented above we are including a copy of the Evaluation Methods Manual. The Statistical Method Summary is found in Section 4, Method #8, page 40, of the manual and is intended to help a scientist understand the basis of the experimental objectives developed for this test.

NETAC

Static Acute Toxicity of Oil Spill Eater II, Batch 329, to the Mysid, *Mysidopsis bahia*

Study Completed

March 9, 1990

Performing Laboratory

EnviroSystems Division
Resource Analysts, Incorporated
P.O. Box 778
One Lafayette Road
Hampton, New Hampshire 03842

I. SUMMARY

The acute toxicity of Oil Spill Eater II, batch 329 to the mysid, *Mysidopsis bahia*, is described in this report. The test was conducted for Incorporated for 96 hours during March 5-9, 1990, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Jeanne Magazu, Peter Kowalski, Robert Boeri, and Timothy Ward.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a mean temperature of 19.5°C. The dilution water was filtered natural seawater collected from the Atlantic Ocean at Hampton, New Hampshire. Aeration was not required to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of Oil Spill Eater II were: 0 mg/L (control), 1 mg/L, 10 mg/L, 100 mg/L, 1,000 mg/L, and 10,000 mg/L. Nominal concentrations were used for all calculations.

Mysids used in the test were less than 5 days old at the start of the test. They were produced at Resource Analysts, Inc. and acclimated under test conditions for their entire life. All mysids were in good condition at the beginning of the study.

Exposure of mysids to the test substance resulted in a 96 hour LC50 of 2,100 mg/L Oil Spill Eater II, with a 95 percent confidence interval of 100 - 10,000 mg/L. The 96 hour no observed effect concentration is estimated to be 100 mg/L.

IV. METHODS AND MATERIALS

TEST SUBSTANCE:

Oil Spill Eater II (EnviroSystems Sample Number 2351E) was delivered to EnviroSystems on March 5, 1990. It was contained in a 500 ml plastic bottle that was labelled with the following information: Oil Spill Eater II, Batch 329. The sample was supplied by Incorporated. Prior to use the test material was stored at room temperature. Nominal concentrations were added to test media on a weight/vol basis and are reported as mg/L.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was seawater collected from the Atlantic Ocean at EnviroSystems in Hampton, New Hampshire. Water was adjusted to a salinity of 11-17 ppt (parts per thousand) and stored in 500-gallon polyethylene tanks, where it was aerated.

TEST ORGANISM

Juvenile mysids employed as test organisms were from a single source and were identified using an appropriate taxonomic key. They were produced and acclimated at the Resource Analysts, Inc. facility for their entire life. During acclimation mysids were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. Mysids were fed newly hatched *Artemia salina* nauplii (EnviroSystems lot number BS01) once or twice daily before the test.

TOXICITY TESTING:

The definitive toxicity test was performed during March 5-9, 1990. It was based on procedures of the U.S. Environmental Protection Agency (1986, 1987). The test was conducted at a target temperature of 20 ± 2°C with five concentrations of test substance and a dilution water control. A stock solution was prepared by combining 20.0 g of test substance with 2,000 ml of dilution water. The stock solution was added directly to dilution water contained in the test vessels without the use of a solvent. Nominal concentrations of the test material were: 0 mg/L, 1 mg/L, 10 mg/L, 100 mg/L, 1,000 mg/L, and 10,000 mg/L.

Twenty mysids were randomly distributed among a single replicate of each treatment. The test was performed in 2 liter glass dishes (approximately 25 cm in diameter and 8 cm deep) that contained 1.0 liter of test solution (water depth was approximately 4 cm). Test vessels were randomly arranged in an incubator during the 96 hour test. A 16 hour light and 8 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of 40 $uEs^{-1}m^{-2}$. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. Mysids were fed newly hatched *Artemia salina* nauplii once per day during the test.

The number of surviving organisms and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) were determined visually and recorded initially and after 24, 48, 72, and 96 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-3), pH (Beckman model pHI 12 meter; instrument number PRL-4), salinity (Labcomp SCT meter, instrument number PRL-6), and temperature (ASTM mercury thermometer; thermometer number 2211) were measured and recorded daily in each test chamber that contained live animals.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques. Computer methods (Stephan, 1983) were used to calculate the 96 hour median lethal concentration (LC50). The no observed effect level is the highest tested concentration at which 90% or more of the exposed organisms were unaffected.

V. RESULTS

No insoluble material was observed in any test vessel during the test. Biological and water quality data generated by the acute toxicity test are presented in Table 1 and Appendix A, respectively. One hundred percent survival occurred in the control exposure.

The dose - response curve for organisms exposed to the test substance for 96 hours is presented in Figure 1. Exposure of mysids to the Oil Spill Eater II, batch 329, resulted in a 96 hour LC50 of 2,100 mg/L, with a 95 percent confidence interval of 100 - 10,000 mg/L. The 96 hour no observed effect concentration is estimated to be 100 mg/L.

Table 1. Survival data from toxicity test

Nominal Concentration (mg/L)		Number Alive					Number Affected				
		0hr	24hr	48hr	72hr	96hr	0hr	24hr	48hr	72hr	96hr
0 (control)	1	10	10	10	10	10	0	0	0	0	0
1	1	10	10	9	9	9	0	0	0	0	0
10	1	10	10	9	9	9	0	0	0	0	0
100	1	10	10	10	9	9	0	0	0	0	0
1,000	1	10	9	9	8	8	0	0	0	0	0
10,000	1	10	0	0	0	0	0	-	-	-	-

Acute, Definitive Toxicity Tests of the Material OSE II to the Mummichog (*Fundulus heteroclitus*)

Toxicity Test Report

Submitted by:

Biomonitoring Services Laboratory
6600 East Bay Boulevard
Gulf Breeze, Florida 32561
(904) 932-2717

Project Number: 52-01-AA467-AA469
Report Number: 52-AA467-469-1-BSL-8-90

August 1990

Toxicity Test Summary Sheet

Client Contact: Carol Wilson

Report Date & Number: August 1990, 52-AA467-469-1-BSL-8-90

Project Number: 52-01-AA467-AA469

Study Director: Dan Johnson/Jamie McKee

Test Material: OSE II alone, #2 Fuel alone, OSE II #2 Fuel Oil

Description: Brownish, odorless liquid

Dates Materials Received: June 1990

Dates of Definitive Test: 28 June through 2 July 1990

Test Conditions: Static, aerated, 96-hour duration

Test Procedure: U.S. Environmental Protection Agency. 1989. National Oil and Hazardous Substance Pollution Contingency Plan; Final Rule. Federal Register, 40CFR Part 300, July 18, pp 29192-29207.

Test Animals: Mummichog (Fundulus heteroclitus); Lot number: FH-90-2

Source: Commercial aquaculture supply company
Size: 1 - 1.5 inches

Dilution/Control Water: Artificial seawater at 20 parts per thousand salinity.

Test Concentrations: 500, 1,000, 2,000, 4,000 and 8,000 parts per million OSE II.

Effect Criterion: Mortality

96-Hour LC50 for OSE II: 5,258.09 parts per million with a 95% confidence interval of 4,000.0 - 8,000.0 per million.

96-Hour LC50 for #2 Fuel Oil: 320.03 parts per million with a 95% confidence interval of 129.19 - 644.39 parts per million.

96-Hour LC50 For OSE II #2 Fuel Oil: 125.00 parts per million with a 95% confidence interval of 0.00 - infinity parts per million.

OIL SPILL EATER INTERNATIONAL, CORP.

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TOXICITY TEST

FOR ARTEMIA SALINA

To gain acceptance on the U.S. EPA's National Contingency Plan List, we were requested to perform an additional Toxicity Test on Artemia Salina using EPA's Standard Dispersant Toxicity Test.

OSE II Concentrate was presented to the laboratory, but the laboratory refers to the product as a Dispersant throughout the write-up since it was a Dispersant Toxicity Test. The Test proved that OSE II Concentrate is once again virtually non-toxic. This particular test proved OSE II helps to detoxify the oil in some organisms. The fuel had a higher toxicity rate than did the fuel and OSE II.

OSE II gained acceptance to the EPA's National Contingency Plan once this test was presented to the EPA.

By: Steven R. Pedigo
Chairman, OSEI, Corp.

SRP/AJL

Standard Dispersant Toxicity Test With The OSE II, Batch #9820 and Artemia salina

AUTHORS

Timothy J. Ward
Robert L. Boeri

PERFORMING LABORATORY

EnviroSystems Division
Resource Analysts, Incorporated
P. O. Box 778
One Lafayette Road
Hampton, New Hampshire 03842

October, 1990

II. TABLE OF CONTENTS

III. INDEX OF TABLES

IV. INTRODUCTION

The objective of the study was to determine the acute toxicity of the dispersant - Batch # 9820, No.2 fuel oil, and a 1:10 mixture of dispersant and oil to *Artemia salina*, a marine invertebrate. The report contains sections that describe the methods and materials employed in the study, and the results of the investigation. The report also contains an appendix that presents the water quality data collected during the tests.

V. METHODS AND MATERIALS

TEST SUBSTANCE:

The dispersant - Batch # 9820 (EnviroSystems Sample Number 2591E) was delivered to EnviroSystems on August 17, 1990. It was contained in two 1,000 ml plastic bottles that were labelled with the following information: "Batch # 9820". The No. 2 fuel oil (EnviroSystems Sample Number 2599E) was delivered to EnviroSystems on August 28, 1990. It was contained in a 1,000 ml plastic bottle that was labelled with the following information: "# 2 fuel oil".

DILUTION WATER:

Water used for hatching and acclimation of test organisms and for all toxicity testing was formulated at EnviroSystems in Hampton, New Hampshire. Water was diluted to a salinity of 20 parts per thousand and stored in polyethylene tanks where it was aerated.

TEST ORGANISM:

Juvenile *Artemia salina* employed as test organisms were from a single source and were identified using an appropriate taxonomic key. *Artemia salina* used in the test were produced from an in-house culture and were 24 hours old at the start of the test. Prior to testing, *Artemia salina* were maintained in 100% dilution water under static conditions. During acclimation *Artemia salina* were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. They were not fed before or during the tests.

TOXICITY TESTING:

Screening tests with the test substances were conducted during October 1 to 3, 1990. The definitive toxicity tests were performed with the dispersant, No. 2 fuel oil, a 1:10 mixture of dispersant and oil, and the standard toxicant, dodecyl sodium sulfate during October 3 to 5, 1990, according to procedures of the U.S EPA (1984). The tests were conducted at a target temperature of 20 ± 1°C with five concentrations of each test substance and a dilution water control.

The dispersant and oil stock solutions were prepared by combining 550 ml of sea water and 0.55 ml of test substance in a glass blender jar and mixing the solution at 10,000 rpm for 5 seconds. The combined dispersant and oil stock solution was prepared by mixing 550 ml of sea water at 10,000 rpm and adding 0.5 ml of oil and 0.05 ml of dispersant. This combined mixture was then mixed for 5 seconds. Nominal concentrations of each test material were: 0 mg/L (control), 10 mg/L, 25 mg/L, 40 mg/L, 60 mg/L, and 100 mg/L. Media in each test vessel was added at the beginning of the test and not renewed.

Twenty *Artemia salina* were randomly distributed to each of 5 replicates of each treatment. The tests were performed in 250 ml glass Carolina culture dishes that contained 100 ml of test solution (water depth was approximately 2.5 cm). Test vessels were randomly arranged in an incubator during the 48 hour test. A 24 hour light and 0 hour dark photoperiod was maintained below the dishes. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. *Artemia salina* were not fed during the tests.

The number of surviving organisms was determined visually and recorded initially and after 24 and 48 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-18), pH (Beckman model pHI 12 meter; instrument number PRL-4), salinity (Refractometer, instrument number PRL-6), and temperature (ASTM mercury thermometer; thermometer number 2211) were measured and recorded at the beginning and end of each test in one test chamber of each concentration.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques (Stephan, 1983). The binomial method was used to calculate the median lethal concentration (LC50) values.

VI. RESULTS

All test vessels containing dispersant appeared clear throughout the test and all test vessels containing oil or oil and dispersant had an oil slick on the surface of the test media throughout the test. Biological and water quality data generated by the acute toxicity tests are presented in Table 1 and Appendix A, respectively. Ninety-nine percent survival occurred in the control exposure. The 48 hour LC50 for *Artemia salina* exposed to the reference toxicant dodecyl sodium sulfate is 38.7 mg/L.

The 24 and 48 hour LC50s from the three toxicity tests are presented in Table 2. The 48 hour LC50s for *Artemia salina* exposed to the test substances are: dispersant - >100 mg/L, No. 2 fuel oil - 12.6 mg/L (95% confidence interval = 10.0 - 25.0 mg/L), and a 1:10 mixture of dispersant and No. 2 fuel oil - 29.4 mg/L (95% confidence interval = 25.0 - 40.0 mg/L).

Table 1. Survival data from toxicity tests

Nominal Concentration (mg/L)	rep.	Number Alive								
		Dispersant			No. 2 fuel oil			Oil + Dispersant		
		0hr	24hr	48hr	0hr	24hr	48hr	0hr	24hr	48hr
0 (control)	1	20	20	20	20	20	20	20	20	20
	2	20	20	19	20	20	19	20	20	19
	3	20	20	20	20	20	20	20	20	20
	4	20	20	20	20	20	20	20	20	20
	5	20	20	20	20	20	20	20	20	20
10	1	20	19	17	20	20	17	20	20	19
	2	20	20	17	20	20	19	20	20	18
	3	20	20	20	20	20	12	20	18	18
	4	20	20	19	20	20	9	20	20	17
	5	20	19	18	20	18	10	20	20	16
25	1	20	20	16	20	18	0	20	19	19
	2	20	19	17	20	19	3	20	18	15
	3	20	20	18	20	19	2	20	20	16
	4	20	19	12	20	20	2	20	20	17
	5	20	19	15	20	20	0	20	19	14
40	1	20	19	16	20	20	0	20	19	0
	2	20	20	14	20	19	0	20	20	0
	3	20	20	19	20	20	0	20	20	0
	4	20	20	15	20	18	0	20	14	0
	5	20	20	17	20	17	0	20	18	2
60	1	20	19	18	20	18	0	20	18	0
	2	20	19	16	20	19	0	20	18	0
	3	20	19	19	20	16	0	20	19	0
	4	20	20	17	20	19	0	20	16	0
	5	20	20	16	20	14	1	20	16	1
100	1	20	20	18	20	13	0	20	20	0
	2	20	20	18	20	8	0	20	20	0
	3	20	19	13	20	9	0	20	20	0
	4	20	20	19	20	10	0	20	20	0
	5	20	20	16	20	8	0	20	20	0

Table 2. Median lethal concentrations (LC50s) from toxicity tests

Test substance	Time	LC50	95 percent confidence limits	Calculation method
Dispersant	24 hour	> 100 mg/L	--	--
	48 hour	> 100 mg/L	--	--
No. 2 fuel oil	24 hour	> 100 mg/L	--	--
	48 hour	12.6 mg/L	10 - 25 mg/L	Binomial
Dispersant and No. 2 fuel oil	24 hour	> 100 mg/L	--	--
	48 hour	29.4 mg/L	25 - 40 mg/L	Binomial

VII. REFERENCES

Stephan, C.E. 1983. Computer program for calculation of LC50 values. Personal communication.

U.S. EPA. 1984. Revised Standard Dispersant Toxicity Test. Federal Register, Volume 49, Number 139, Wednesday, July 18, 1984, pages 29204 to 29207.

Appendix A. WATER QUALITY DATA FROM TOXICITY TESTS

I. SUMMARY

The acute toxicity of the dispersant - Batch #9820, No. 2 fuel oil, and a 1:10 mixture of dispersant and No. 2 fuel oil to Artemia salina, is described in this report. The test was conducted for corp for 48 hours during October 3 to 5, 1990, at the Envirc-Systems Division of Resource Analysts, Inc. in Hampton, New Hampshire.

The test was performed under static conditions with five concentrations of each test substance and a dilution water control at a temperature of 20 = 1°C. The dilution water was sea water adjusted to a salinity of 20 parts per thousand. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of all three test substances were: 0 mg/L (control), 10 mg/L, 25 mg/L, 40 mg/L, 60 mg/L and 100 mg/L. Nominal concentrations were used for all calculations.

Artemia salina used in the test were 24 hours old at the start of the test and they were all in good condition at the beginning of the study. Exposure of Artemia salina to the test substances resulted in the following 48 hours median lethan concentrations (LC50): dispersant-100 mg/L, No. 2 fuel oil - 12.6 mg/L (95% confidence interval = 10.0-25.0 mg/L), and a 1:10 mixture of dispersant and No. 2 fuel oil-29.4 mg/L (95% confidence interval = 25.0 - 40.0 mg/L).

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SUMMARY

ENVIRONMENT CANADA'S TOXICITY TEST

Environment Canada performs Toxicity Testing for determining if a product could gain approval for use in Canada. The level that is considered toxic is 1,000 mg/L or less. A product that exceeds this level is deemed acceptable.

OIL SPILL EATER II Concentrate, tested at 10,000 mg/L - which shows OSE II Concentrate is virtually non-toxic and far exceeds the level deemed to toxic by Environment Canada.

Rainbow Trout is one of the most sensitive fresh water organisms to test. OSE II proved that even with third party testing by a Foreign Government, OSE II is virtually non-toxic.

By: Steven R. Pedigo
Chairman/OSEI, Corp.

Environment Canada | Environnement Canada
Conservation and Protection | Conservation et Protection
Emergencies Science Division
River Road Environmental Technology Centre
3439 River Road
Ottawa, Ontario K1A 0H3

CANADA'S GREEN PLAN / LE PLAN VERT DU CANADA

Your file / Votre référence

Our file / Notre référence
4808-13-7

May 17, 1993

Steven R. Pedigo, Chairman,
OSEI Corporation
5545 Harvest Hill
Suite 1116
Dallas, Texas 75230
U.S. A.

Dear Mr. Pedigo,

Thank-you for participating in the development of Environment Canada's draft guidelines for assessing the toxicity and effectiveness of oil spill bioremediation agents (OSBAs).

The Tier I toxicity testing is now complete. Our preliminary screening has indicated that the *Daphnia magna* test and the Microtox test were either insensitive or erratic. Therefore, we do not consider these particular tests useful for OSBA evaluation. Comments on the toxicity of your product will thus be limited to those obtained using the 96-hour Rainbow Trout acute lethality test. 'Oil Spill Eater II' had a rainbow trout 96-hour LC50 of greater than 10,000 mg of application solution per litre of water. There was, however, a 23% mean fish mortality at this concentration. Also note that between 24 and 96 hours of exposure to the product, sublethal effects were present. The fish were noted to surface, be on their side, turn dark, exhibit rapid breathing and no swimming. These sublethal effects should be of concern. The effectiveness test analyses are still being performed. You will be notified as soon as those results are available.

If your product meets both the effectiveness and toxicity criteria it will be placed on our Standard List of Oil Spill Bioremediation Agents. Placement on this list is not an indication that the product will be used in the event of an oil spill. The list and test results are public information. They may be provided to oil spill response personnel to enable them to make informed decisions.

Please take note that *the placement of a product on our Standard List does not constitute an approval or certification or licensing of your product for use in Canada.* Your product may be required to comply with the New Substances Notification Regulations (NSNR) for biotechnology products under the Canadian Environmental Protection Act (CEPA). For information on the draft regulations, please contact the Chief of the New Substances Division at (819) 997-4336 or at the following address: Chief, New Substances Division, CCB, Environment Canada, P.V.M. 14th Floor, Ottawa, Ontario K1A 0H3, CANADA.

Sincerely,

Merv Fingas
Chief, Emergencies Science Division

Encl.

ENVIRONMENT CANADA

TIER I TOXICITY TESTING

FOR EVALUATION OF DRAFT OSBA GUIDELINES

The testing was performed as follows. An application solution of the OSBA was prepared based on instructions provided by the manufacturer/supplier. The highest strength of solution tested was 10,000 mg of application solution per litre of water (approx. a 1:100 dilution). For products in which solids are normally added to the water, suspensions comprised of 10,000 mg of product/combined product per litre of water were prepared for use in the toxicity tests. (If several solids were to be added, they were combined in the appropriate ratio). This initial screening concentration was tested in triplicate. If this concentration was toxic to greater than 50% of the organisms, lower concentrations were tested. Sub-lethal effects on the behavior and/or appearance of the organisms were also made. The toxicity of the product in water was assessed using each of the following three biological test methods, developed and standardized by Environment Canada for these and other applications:

Environment Canada, 1990a. **Biological test method: acute lethality test using rainbow trout**. Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/9, 51 pp.

Environment Canada, 1990b. **Biological test method: acute lethality test using *Daphnia* spp.** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/11, 57 pp.

Environment Canada, 1992. **Biological Test method: toxicity test using luminescent bacteria (*Photobacterium phosphoreum*)**. Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/24, 61 pp.

May 17, 1993

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TOXICITY TEST SUMMARY USING

CITGO GASOLINE, OIL SPILL EATER II

AND FATHEAD MINNOWS

To prove OIL SPILL EATER II rapidly detoxifies hydrocarbons once OSE II is applied, a Toxicity Test was set up with the Physical Engineer of the City of Plano, Texas.

One half gallon of gasoline was poured onto a concrete surface, where 1/2 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 was used to wash this effluent into a catch basin. Approximately 1 1/2 gallons was 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 respons4e 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.

By: Steven R. Pedigo
Chairman/OSEI, Corp.

OSEI CORPORATION

OSE II/GASOLINE/WATER

Toxicity Test Report

DECEMBER 7, 1991

BIO-AQUATIC TESTING, INC.

Prepared by: *David Smith,*
Aquatic Toxicologist

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
Sample . . . . . OSE II/Gasoline/Water

Laboratory I.D. **BO-12-91-2239**
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

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*).

CHEMICAL MEASUREMENTS

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.

TEST PROCEDURES
Pimephales promelas

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.

LC50 RESULTS
Pimephales promelas

LC50 value calculated using the Binomial Method:

CONC. (mg/L)	# EXPOSED	# DEAD	% DEAD	BINOMIAL %
30,000	20	20	100	0.0001
9,000	20	6	30	5.7659
3,000	20	1	5	0.0020
800	20	0	0	0.0001
200	20	0	0	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-Karber 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

SUMMARY

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
SAMPLE OSE II, Gasoline, Water
LAB ID # BO-12-91-2239B
DATE COLLECTED 12/05/91
DATE RECEIVED 12/05/91
SAMPLE TYPE Grab
TEST TYPE Acute

BEGIN DATE 12/06/91
END DATE 12/08/91
TEST ORGANISM *Pimephales promelas*
TEST TEMPERATURE (°C) 25° ± 1
PHOTO PERIOD 16 hour light / 8 hour dark
LIGHT INTENSITY 75 FT-C
ANALYST W. Smith

SURVIVAL SUMMARY

% EFFLUENT CONC	NUMBER LIVE PER REP												$\bar{x}$ LIVE PER CONC
	START				24 HOURS				48 HOURS				
	a	b	c	d	a	b	c	d	a	b	c	d	$\bar{x}$ % Surv.
Control	5	5	5	5	5	5	5	5	5	5	5	5	100
200 mg/L	5	5	5	5	5	5	5	5	5	5	5	5	100
800 mg/L	5	5	5	5	5	5	5	5	5	5	5	5	100
3,000 mg/L	5	5	5	5	5	5	5	5	5	4	5	5	95
9,000 mg/L	5	5	5	5	3	3	5	5	3	1	5	5	70
30,000 mg/L	5	5	5	5	0	0	0	0	0	0	0	0	0

EFFLUENT MEASUREMENTS

D.O. @ 30,000 mg/L[1] 8.6/6.6
pH @ 30,000[1] 8.3/8.4
CONDUCTIVITY @ 30,000 (μMHOS) 500
HARDNESS (mg/L as $CaCO_3$) 272.4 ALKALINITY (mg/L as $CaCO_3$) 625.0

DECHLORINATION

RESIDUAL Cl_2 (mg/L) <0.10 ANALYSIS METHOD Amperometric Titration Method (330.1)
DECHLORINATION REAGENT Not Applicable

DILUTION WATER MEASUREMENTS

D.O. @ 100% (mg/L)[1] 8.6/6.9
pH @ 100%[1] 8.4/8.3
RECEIVING WATER DILUTION WATER Laboratory adjusted
HARDNESS (mg/L as $CaCO_3$) 160.0 ALKALINITY (mg/L as $CaCO_3$) 107.0

[1] Recorded at the beginning and end of each 24-hour exposure period.

I. SUMMARY

The acute toxicity of the dispersant - Batch #9820, No. 2 fuel oil, and a 1:10 mixture of dispersant and No. 2 fuel oil to Artemia salina, is described in this report. The test was conducted for corp for 48 hours during October 3 to 5, 1990, at the Enviro-Systems Division of Resource Analysts, Inc. in Hampton, New Hampshire.

The test was performed under static conditions with five concentrations of each test substance and a dilution water control at a temperature of 20 = 1°C. The dilution water was sea water adjusted to a salinity of 20 parts per thousand. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of all three test substances were: 0 mg/L (control), 10 mg/L, 25 mg/L, 40 mg/L, 60 mg/L and 100 mg/L. Nominal concentrations were used for all calculations.

Artemia salina used in the test were 24 hours old at the start of the test and they were all in good condition at the beginning of the study. Exposure of Artemia salina to the test substances resulted in the following 48 hours median lethan concentrations (LC50): dispersant-100 mg/L, No. 2 fuel oil - 12.6 mg/L (95% confidence interval = 10.0-25.0 mg/L), and a 1:10 mixture of dispersant and No. 2 fuel oil-29.4 mg/L (95% confidence interval = 25.0 - 40.0 mg/L).

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BOD COD SUMMARY
(Biological Oxygen Demand / Concentrated Oxygen Demand)

When a given area receives or becomes contaminated with a given carbon based contaminate the BOD/COD is automatically affected!

Oil Spill Eater II **(OSE II)** in and of itself only slightly affects BOD/COD regardless of the application rates of OSE II. The effect of using OSE II would, at most, be 5% to 10% on the BOD.

In any area where there is water movement or tidal action, the BOD/COD uptakes effects would be minimal to the alternative of leaving an untreated contaminant in place where it could potentially affect the BOD/COD or harm waterfowl, birds, mammals, fish and plant life.

The potential of long-term problems of leaving a contaminant in place should be of more concern than minutely affecting the BOD/COD by using OSE II.

In our experience, BOD and COD problems really only need to be addressed where you want to treat a contaminant in a closed system or a small body of water where there is no inflow of water. Even in these systems, the BOD/COD can be maintained simply by pumping air into the system or pumping the water into the air, or by causing an inflow of water to the area that has become contaminated.

Oil Spill Eater II was used on a 3-acre pond with fish and wildlife swimming in the water where approximately 1 1/2 acres of the pond was covered with crude oil from a pipeline break. We applied our product on the shoreline to remove the crude oil from the grasses, plant life and marsh area. OSE II was then applied to the main body of the spill. A circulation pump was set out in the middle of the pond where water was pumped up in the air. There were fish, snakes and turtles observed swimming in the water away from the spill and no fish or wildlife died. It took 3 days for bacteria growth to be visible to the human eye and in 5 days visible clean patches started appearing in the crude oil where the bacteria was converting the oil to CO_2 and water.

SUMMARY

BOD/COD concerns where there is an open system is minimal, compared to long term problems of leaving a contaminant in place untreated. If you want to or feel addressing the BOD/COD problem is needed, then pumping air into the area or moving the water is easily performed and should be attempted over leaving an untreated contaminant in place.

The RRT/Onscene Commanders require even one gallon spills to be reported and mechanically cleaned up. How can they authorize leaving a large spill (25,000 gallons) in place and untreated. If there is enough contaminate to adversely affect the BOD/COD in any eco system, then the contaminant itself would choke the life out of everything.

We would think that you would want to return any given eco system to it's pre-spill conditions as fast as possible by utilizing a product such as OSEI.

BY: Steven R. Pedigo
Chairman

SRP/AJL

To Whom this may concern,

re: OSEI's product for petroleum hydrocarbon remediation in aquatic environments.

This report is in response to concerns expressed by U.S. EPA regulatory officials about the use of OSEI's product in surface waters for remediation of petroleum hydrocarbon spills. I understand that this concern is for the potential increase in biochemical oxygen demand (BOD) as a result of administering OSEI's product to remediate contaminated water. My research over the last several years has been involved in testing various aeration and management techniques used to overcome severe oxygen depletion in the hypolimnion of eutrophic lakes. I have even evaluated the use of Bact-A-Pur® for its potential to reduce sediment organic matter. Specific goals have included remedial practices for winterkill prevention, maintaining an oxidized microzone at the sediment surface to minimize dissolution of iron, manganese, sulfides, reduced organic acids and methane into the water column of eutrophic lakes. In performing these tests it has been necessary to isolate, measure and model sources of oxygen depletion including sediment chemical and biological oxygen demand, phytoplankton respiration and methanogeneses in anaerobic sediments. This research has culminated in the completion of a Ph.D. thesis under the direction of W.C. Mackay and Dave Schindler at the University of Alberta and several publications have been submitted or are currently being written concerning this aspect of limnology. Further, I was indirectly involved in but have extensively reviewed the data and discussed the results of bioremediation testing with the experts involved in the Exxon Valdez disaster in Alaska.

After review of information provided to me by George Lively, President of OSEI, Inc. I have the following comments.

Bioremediation, and specifically the OSEI product, is undoubtedly an effective and inexpensive approach for the remediation of petroleum hydrocarbon spills. In addition, although the efficacy of earlier tests for bioremediation products in rivers ans streams was questionable the OSEI product particularly appears to emulsify, and maintain the oil at the surface as it proceeds to degrade the spill. This characteristic is particularly beneficial in its use in lentic systems and has and will continue to prove to be an ideal application of this new technology. Specifically, there are several factors which should be pointed out which support this position and explain why this application will have minimal or no impact on the BOD in lentic aquatic systems.

1. The specific species of bacteria which the enzyme and nutrient solution are designed to target are but a tiny minority of the aerobic bacterial community of freshwater and marine ecosystems. Hence, there will be only a minuscule increase in the overall bacterial community with a concomitant minuscule (although not likely measurable), increase in BOD.

The small addition of nutrients may, however, temporarily enhance the phytoplankton population in very small bodies of water.

2. This possibility would be even further reduced for a hydrocarbon spill in freshwater or coastal wetlands. This is because theses systems are inherently hypereutrophic and hence already possess large amounts of organic matter with associated high rates of BOD. (I have observed such water bodies to range in DO from > 15 mg L^{-1} in mid-afternoon to 0 mg L^{-1} for several pre-dawn hours). Hence, an additional small amount of BOD would likely neither be observed nor have any

additional ecological impacts to the present system. Further, the small nutrient additions will likely not exceed background values for nitrogen and phosphorus in these productive systems

3. One of the greatest merits of this product is that, because the oil-degrading bacteria use only petroleum hydrocarbons as substrates, these populations will diminish to pre-spill low abundance once hydrocarbons are oxidized. Hence, after just a few weeks of treatment the aquatic ecosystem will revert to pre-spill conditions.

4. Even an accidental excessive dose of the OSEI product would have no toxicological consequences and would result only in a minor and temporary increase in nutrients and possible phytoplankton growth. In comparison with other remediation techniques which require dredging, pumping and treating or air stripping, the use of this product is much cheaper, incurs minimal collateral ecological damage and leaves no physical, toxicological or ecological impairment.

T. G. Miller

Theron G. Miller
President, Aquatic Solutions, LLC

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TEST ON MOBILIZING

PRUDHOE BAY CRUDE

Performed by Steve Hinton - Exxon U.S.A.

A Shaker Flask Test was performed using Prudhoe Bay Crude and "OIL SPILL EATER II" mixed 50 parts seawater to One (1) part OSE II.

Rocks covered with 400 grams of Prudhoe Bay Crude were coated with 400 ml of diluted "OIL SPILL EATER II". Steve Hinton at Exxon claimed that OSE II mobilized all the Prudhoe Bay Crude in about 6 to 8 hours.

This shows OSE II is very effective in cleaning oil off of rocks and was proven by Exxon.

Test was performed on January 4th and 5th, 1990.

Steven R. Pedigo
Chairman

SRP/AJL

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April 23, 1990

Percolation Rate of
Oil Spill Eater

1. Objective of experiment was to determine the depth of percolation of Oil Spill Eater the rate of percolation involving several potential beach materials.

2. 4 clear troughs 24" L x 18" W x 10" D were used with ¼" markings on all sides.

   A. Trough number 1, had a sand and gravel mixture of ¼" sand and ½" ⅛" gravel 6" deep. It was placed on a screen held 1" above the bottom of the trough. Screen contained slits that were 5 microns in size.

   B. Trough number 2, had a predominately gravel base with a small amount of sand mixed in. Gravel was 1" rock diameter, and this gravel was placed on a screen held 1" above the bottom of the trough. Screen contained slits that were 5 microns in size.

   C. Trough number 3, used small boulders of 6" in diameter. Boulders were placed on a screen held 1" above the bottom of the trough. Screen contained slits that were 5 microns in size.

   D. Trough number 4, a mixture of 1" gravel and 6" boulders were placed on a screen held 1" above the bottom of the trough. The screen contained slits that were 5 microns in size. The screen was covered with a 2" layer of 1" rock. The 2" layer of rock was then coated with an ⅛" of an inch of Prudoe Bay Crude.

3. All 4 troughs material was saturated with Alaskan sea water that was 40°f. Red dye was mixed with OSE to visually observe percolation depth.

5. A ¼" of an inch layer of OSE was then applied and visual data was then noted.

PERCOLATION TEST

Results

Once of the volume of the 50 to 1 OSE had percolated to the bottom of the trough, then time was noted. Depth of Red Dye was used as a leading edge indicator.

A. Trough number 1, OSE percolated quickly to 4" then slowed somewhat, and at 47 minutes of the original OSE volume was measured in the bottom of the trough.

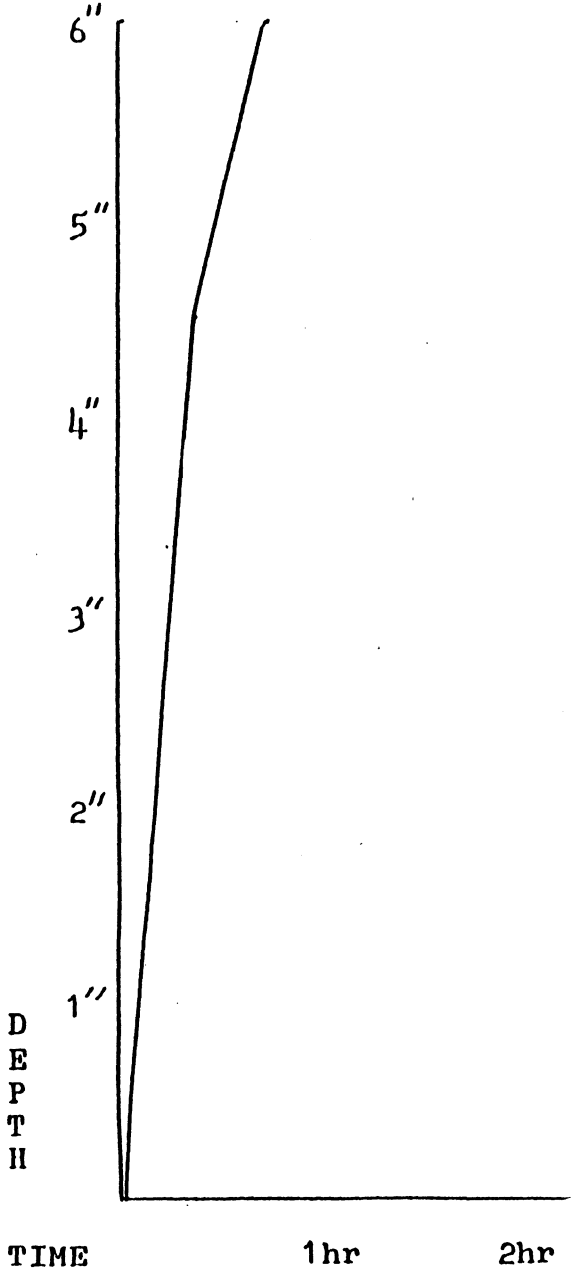
B. Trough number 2, OSE percolated quickly to 4½ inches and still proceeded quickly and at 28 minutes, of the original OSE volume was measured in the bottom of the trough.

C. Trough number 3, OSE percolated to the bottom of the trough about as fast as it was applied.

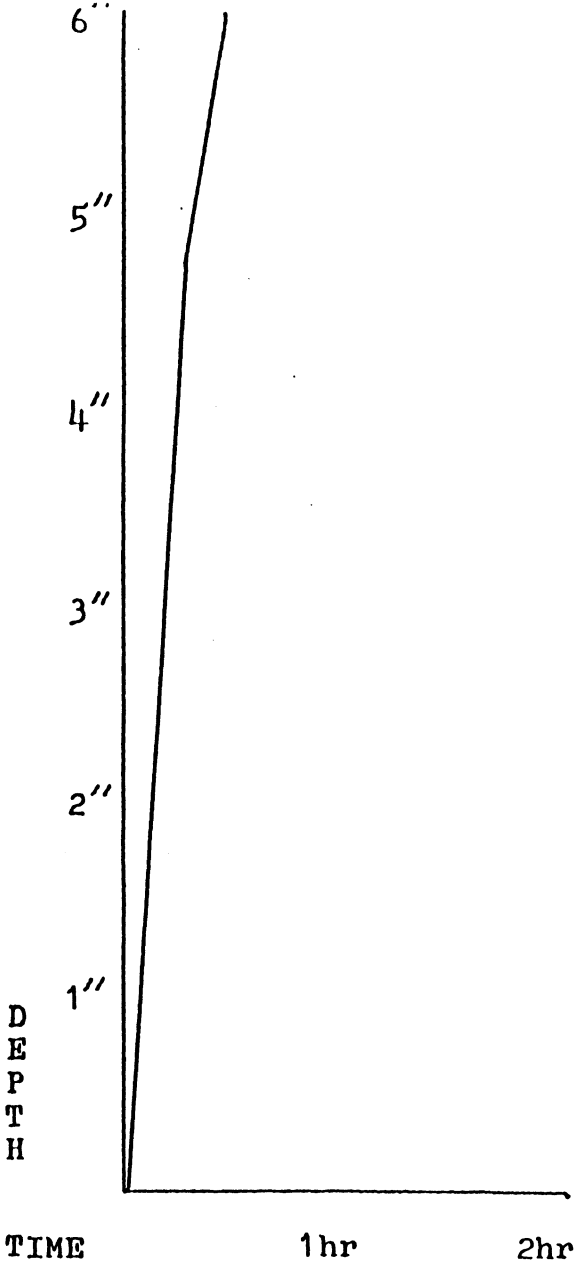
D. Trough number 4, OSE percolated almost as fast as it was applied to the depth of the 1" rocks coated with the Alaskan crude. Once OSE reached the point of contact with the crude, percolation slowed substantially. What was reaching the bottom of the trough was OSE and the Alaskan crude. In 3 hours and 53 minutes, a volume of of the original OSE volume was noted. However, this may not have been precise since the crude that was being mobilized and percolating may have made up some of this volume. Percolation may have slowed due to the OSE adhering to the crude, then mobilizing the crude and then this mixture percolated slower.

Tests were preformed by Steven R. Pedigo.

Steven R. Pedigo
Chairman



TROUGH #1



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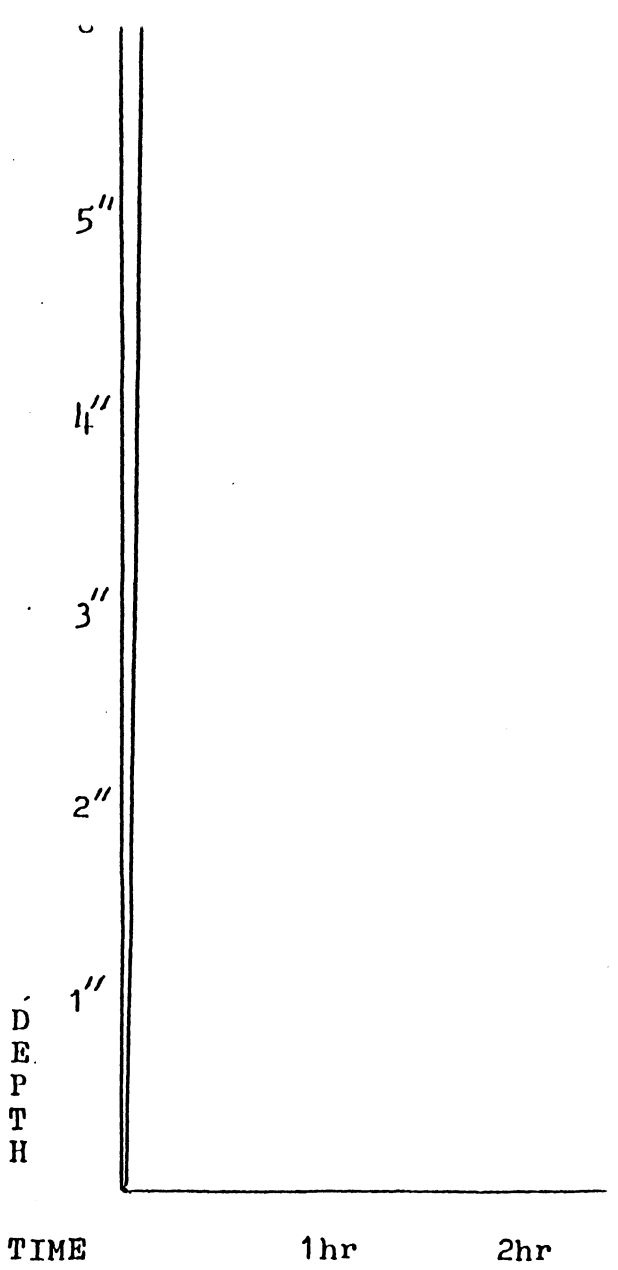
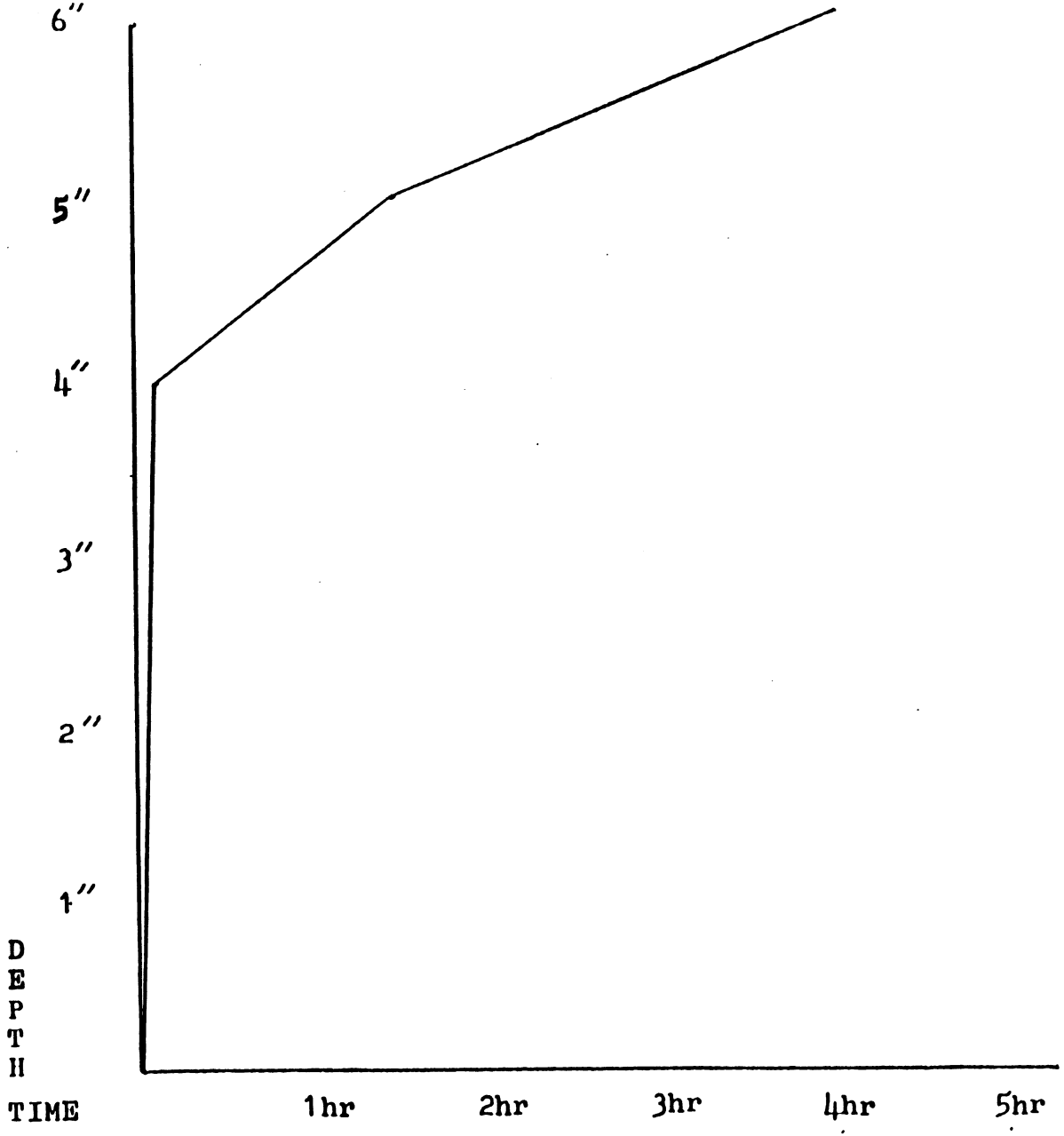


TROUGH #3

PERCOLATION RATES



TROUGH #4 PERCOLATION RATE

OIL SPILL EATER INTERNATIONAL, CORP.

13127 Chandler Drive
Dallas, Texas 75243

(972) 669-3390
(972) 644-8359 (Fax)

June 23, 1999

"OIL SPILL EATER" II (OSE II)

SWIRLING FLASK DISPERSANT EFFECTIVENESS TEST

The attached test by the Southwest Research Institute in San Antonio shows the following information as to the effectiveness of "OIL SPILL EATER" II as a Dispersant for oil:

Note: Time of test is 30 minutes.

1. Percent of Dispersed Oil due to OSE II:

   Effectiveness = 0%

2. Percent Dispersed Oil without OSE II:

   Effectiveness = 62%

3. Since the molecular weight of the crude oil tested is many times that of diesel, jet fuel, or gasoline, OSE II will prevent these lighter oils from sinking into the water column.

4. When you have a spill and have to wait for the clean-up crew or contractor to rig booms and skimmers, the elapsed time could be hours. This would allow a greater percentage of the oil to sink into the water column.

5. By immediately applying "OIL SPILL EATER" II to the spill, you:

   A. Keep the oil out of the water column.

   B. Eliminate the fire hazard.

   C. Protect the Eco-System.

   D. Get rid of the oil through Bioremediation (turns into CO_2 and water).

O. A. (George) Lively
President

OAL/AJL

SOUTHWEST RESEARCH INSTITUTE

6220 CULEBRA ROAD • POST OFFICE DRAWER 28510 • SAN ANTONIO, TEXAS, USA 78228-0510 • (210) 684-5111 • TELEX 244846

PETROLEUM PRODUCTS TEST REPORT

June 22, 1999

George Lively
Oil Spill Eater International, Corp. (OSEI)
13127 Chandler Drive
Dallas, TX 75243
(972)-669-3390
(972)-644-8359 FAX

RE: Swirl Flask Dispersant Effectiveness Test

SwRI Project Number: 08-2326-088 Workorder: 8783

Dear Mr. Lively:

The "Oil Spill Eater II Concentrate (OSEI)" sample you submitted for Swirl Flask Dispersant Effectiveness Test has as been completed. We received the 8-oz glass jar in good condition on June 8, 1999. The test results are summarized in the attached test report.

Test aliquots were taken in accordance with the manufacturer-suggested procedure. Test conditions are outline in Federal Register/ Volume 59. Testing was performed in accordance with the test procedure used with on deviation or modifications. The analyses pertain only to the sample received by Southwest Research Institute and represent only a sampling of a batch. This report shall not be reproduced exempt in full without the express written permission of Southwest Research Institute.

If we may be of further assistance, or if there are any questions concerning this analysis, please contact me at (210) 522-2024.

Sincerely,

Rose Hill Ward
Research Scientist
Petroleum Products Research Department
Automotive Products and Emissions Research Division

SAN ANTONIO, TEXAS
HOUSTON, TEXAS • DETROIT, MICHIGAN • WASHINGTON, DC

PETROLEUM PRODUCTS TEST REPORT

Oil Spill Eater II Concentrate (OSE II)

Swirl Flask Dispersant Effectiveness Test

TEST	RESULTS	DATE TESTED
% Dispersed with no Dispersant (OSE II)	$EFF_c=(C_{mean}/C_{tot})*100$ **$EFF_c=62.00\%$**	6/21/99
% Dispersed oil with dispersant (OSE II) added	$EFF_a=(C_{mean}/C_{tot})*100$ **$EFF_a=14.56\%$**	6/21/99
% Dispersed oil due to dispersant (OSE II) only	$EFF_D= EFF_a- EFF_c$ $EFF_D=14.56\%-62.00\%$ **$EFF_D=0.00\%$**	6/21/99

Two standards used for this testing were Prudhoe Bay and South Louisiana Reference oils and were obtained through Resource Technology Corporation. The standards were used to determine the response factor.

The OSEI II test dilution is a 1:10 ratio.

The "% Dispersed Oil with no dispersant (OSE II)" contained only the reference oil. The control was used to determine the maximum amount of oil that would naturally leach into the synthetic seawater.

The '% Dispersed oil with dispersant (OSE II) added" contained the oil and an OSEI II as dispersant to determine the effectiveness of the dispersant.

The "% Dispersed Oil due to dispersant (OSE II) only" was a calculated average of eight determinations (four replicates from the South Louisiana reference oil and four replicates from the Prudhoe Bay reference oil).

The results of this testing determine that the OSE II product is totally **ineffective** dispersant according to "Swirling Flask Dispersant Effectiveness Test."

s:\users\rward\oseiafv9.rep

SOUTHWEST RESEARCH INSTITUTE

6220 CULEBRA ROAD • POST OFFICE DRAWER 28510 • SAN ANTONIO, TEXAS, USA 78228-0510 • (512) 684-5111 • TELEX 244846

February 19, 1991

Sky Blue Chemicals
P. O. Box 866412
Plano, TX 75086

Attention: Mr. Steven R. Pedigo

Subject: Analysis of Oil-eater Sample for Contaminants
SWRI 01-3108-092

Dear Mr. Pedigo:

The oil-eater sample received in our laboratory on November 30, 1990, has been analyzed for lead cadmium and total chlorinated hydrocarbons as directed in my conversation with Norman Gouloy of Sign Tech on November 26, 1990. The results of these analyses are shown in the data table which was faxed to you on February 15, 1991. This table is enclosed.

If you have any questions, please call me at (512) 522-2181. Thank you for the opportunity to be of service to your firm.

Sincerely,

Mary R. Riddle

Mary Riddle
Research Scientist

Approved:

Donald E Johnson

Donald E. Johnson, Ph.D.
Director

SKY BLUE CHEM
SwRI PROJECT 01-3108-092

SAMPLE ID: OIL EATER

RESULTS

Analyte	Amount Detected
Pb (mg/kg)	0.8
Cd (ug/kg)	<0.1
Total Chlorinated Hydrocarbons	<5.0 ppm

RAI

Resource Analysts, Inc.
Subsidiary of MILLIPORE
P.O. Box 778, One Lafayette Road
Hampton, N.H. 03842
(603) 926-7777

Mr. Tim Ward
EnviroSystems, Incorporated
P.O. Box 778
Hampton, NH 03842

P.O. Number: ESI 2473E
Date Received: 06/06/90 (1130)
Lab Number: 22,118
Date Reported: 06/15/90

Parameter: Total Cyanide (mg/L) Matrix: Water
Date Analyzed: 06/12/90
Method/Reference: 335.2/40 CFR Part 136, Friday, October 26, 1984

Field Identification:	Laboratory Number	Concentration
Oil Spill Eater II, Batch 9531	22118-1	<0.01

Technical Director

6/15/90
Date

RAI

Resource Analysts, Inc.
Subsidiary of MILLIPORE
P.O. Box 778, One Lafayette Road
Hampton, N.H. 03842
(603) 926-7777

Mr. Tim Ward
EnviroSystems, Incorporated
P.O. Box 778
Hampton, NH 03842

P.O. Number: 2473E
Date Received: 05/25/90 (1415)
Lab Number: 21,986
Date Reported: 06/11/90

Attached please find test results for acid/base/neutral extractable organic compounds.

Field Identification: OSE BATCH 9522 Matrix: Water
Laboratory Number: 21986-1

Parameter	Concentration	Date Analyzed	Method/Ref.
Arsenic, total (mg/L)	<0.01	05/29/90	7060/1
Cadmium, total (mg/L)	<0.005	05/29/90	3010,6010/1
Chromium, total (mg/L)	<0.01	05/29/90	3010,6010/1
Copper, total (mg/L)	0.04	05/29/90	3010,6010/1
Mercury, total (mg/L)	<0.0003	05/30/90	7470/1
Nickel, total (mg/L)	<0.03	05/29/90	3010,6010/1
Lead, total (mg/L)	<0.005	05/29/90	3020,7421/1
Zinc, total (mg/L)	0.06	05/29/90	3010,6010/1

References: 1) EPA SW 846, 3rd Edition

Technical Director

6/11/90
Date

Laboratory number: 21986 -001
Sample Designation: OSE BATCH 9522
Date Analyzed: 06/01/90
Matrix: LIQUID

ACID/BASE/NEUTRAL EXTRACTABLES	CONCENTRATION (ug/L)	DETECTION LIMIT (ug/L)
Phenol	BDL	130
Aniline	BDL	130
Bis(2-chloroethyl)ether	BDL	130
2-Chlorophenol	BDL	130
1,3-Dichlorobenzene	BDL	130
1,4-Dichlorobenzene	BDL	130
Benzylalcohol	BDL	130
1,2-Dichlorobenzene	BDL	130
2-Methylphenol	BDL	130
Bis(2-chloroisopropyl)ether	BDL	130
4-Methylphenol	BDL	130
N-Nitroso-di-N-propylamine	BDL	130
Hexachloroethane	BDL	130
Nitrobenzene	BDL	130
Isophorone	BDL	130
2-Nitrophenol	BDL	130
2,4-Dimethylphenol	BDL	130
Benzoic acid	BDL	630
Bis(2-chloroethoxy)methane	BDL	130
2,4-Dichlorophenol	BDL	130
1,2,4-Trichlorobenzene	BDL	130
Naphthalene	BDL	130
4-Chloroaniline	BDL	130
Hexachlorobutadiene	BDL	130
4-Chloro-3-methylphenol	BDL	130
2-Methylnaphthalene	BDL	130
Hexachlorocyclopentadiene	BDL	130
2,4,6-Trichlorophenol	BDL	130
2,4,5-Trichlorophenol	BDL	630
2-Chloronaphthalene	BDL	130
2-Nitroaniline	BDL	630
Dimethylpthalate	BDL	130
Acenaphthylene	BDL	130
2,6-Dinitrotoluene	BDL	130

ACID/BASE/NEUTRAL EXTRACTABLES	CONCENTRATION (ug/L)	DETECT LIM (ug/
3-Nitroaniline	BDL	63
Acenaphthene	BDL	13
2,4-Dintrophenol	BDL	63
4-Nitrophenol	BDL	63
Dibenzofuran	BDL	13
2,4-Dinitrotoluene	BDL	13
Diethylphthalate	BDL	13
4-Chlorophenyl-phenylether	BDL	13
Fluorene	BDL	13
4-Nitroaniline	BDL	63
4,6-Dinitro-2-methylphenol	BDL	63
N-Nitrosodiphenylamine	BDL	13
Azobenzene	BDL	13
4-Bromophenyl-phenylether	BDL	13
Hexachlorobenzene	BDL	13
Pentachlorophenol	BDL	63
Phenanthrene	BDL	13
Anthracene	BDL	13
Di-N-butylphthalate	BDL	13
Fluoranthene	BDL	13
Benzidine	BDL	63
Pyrene	BDL	13
Butylbenzylpthalate	BDL	13
3,3'-Dichlorobenzidine	BDL	25
Benzo(A)anthracene	BDL	13
Chrysene	BDL	13
Bis(2-ethylhexyl)phthalate	BDL	13
Di-N-octylphthalate	BDL	13
Benzo(B)fluoranthene	BDL	13
Benzo(K)fluoranthene	BDL	13
Benzo(A)pyrene	BDL	13
Ideno(1,2,3,-CD)pyrene	BDL	13
Dibenz(A,H)anthracene	BDL	13
Benzo(G,H,I)perylene	BDL	13

METHOD REFERENCE: 40 CFR PART 136, FRIDAY , OCTOBER 26, 1984
METHOD 625

BDL = Below detection limit

Detection limit raised by the presence of non-listed compounds.

OIL SPILL EATER INTERNATIONAL, CORP.

13127 Chandler Drive
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(972) 644-8359 Fax
admiralgeorge@juno.com

OIL SPILL EATER II
February 12, 2001
TESTING LIGHT-END HYDROCARBONS ON WATER

Bioremediation Test Procedure for:
Diesel Fuel, Jet Fuel, Gasoline, etc.

Materials Needed:

1. 3 liters of natural, fresh or ocean water.
2. OSE II
3. 2 liter wide-mouth beaker.
4. Small aquarium air bubbler.
5. Light-end hydrocarbons.
6. Hand spray aspirator (32 ounces)

Procedure:

1. Make a solution containing 2 ounces of OSE II in 128 ounces (one gallon) of natural, fresh or ocean water. This becomes your OSE II solution.
2. Put 1 liter of natural, fresh, or ocean water in the 2 liter wide-mouth beaker.
3. Add 100 ml of lighten-ends hydrocarbons to the water.
4. Remove 100 ml of the oil and water solution from the beaker. Test for initial contamination level.
5. Since the spill quantity of light hydrocarbons is known (100 ml), apply 100 ml of the OSE II solution to the beaker using a hand sprayer. Spray the outer edges first, working your way to the middle of the light end hydrocarbons. This application will provide 1 part OSE II to 100 parts water to 100 parts light-end hydrocarbons on water which is recommended in the OSE II literature.
6. Turn on aerator (bubbler).
7. At time intervals of initial 0 day, 3 days, 7 days and 15 days after application of OSE II, remove 100 ml samples of test water for analysis. The remaining water can be sampled at any additional time, should 15 days prove inadequate for complete degradation of hydrocarbons.
8. Perform EPA Tests 8015 and 8020 to determine degradation.

SRP/AJL

By: Steven R. Pedigo
Chairman

OIL SPILL EATER INTERNATIONAL, CORP.

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admiralgeorge@juno.com

OIL SPILL EATER II
February 14 , 2001
TESTING - HEAVY-END HYDROCARBONS ON WATER

Bioremediation Test Procedures for:
Crude Oil, Hydraulic Fluid, Motor Oil, Radiator Fluid,
Chlorinated Hydrocarbons, Etc.

Materials Needed:

1. 3 liters of natural, fresh or ocean water.
2. One half pint of OSE II Concentrate.
3. One 2 liter wide-mouth beaker.
4. Small Aquarium (fish tank) with Bubbler for aeration.
5. Heavy- end hydrocarbons.
6. Hand spray aspirator (32 ounces).

Procedure:

1. Make a solution containing 3 ounces of OSE II in 128 ounces (one gallon) natural, fresh, or ocean water. This becomes your OSE II Solution.
2. Put 1 liter of natural, fresh or ocean water in the 2 liter, wide mouth beaker.
3. Add 100 ml of the heavy end- hydrocarbon to be tested to the water.
4. Remove 100 ml of the oil and water solution from the beaker. Test for initial contamination level.
5. Since the spill quantity of heavy- end hydrocarbons is known (100 ml), apply 100 ml of the OSE II mixed solution to the beaker using a hand sprayer. Spray the outer edges, first working your way to the middle of the heavy- end hydrocarbons. This application will provide 2 parts OSE II to 100 parts water to 100 parts heavy-end hydrocarbon on water. This is recommended in our OSE II literature.
6. Turn on Bubbler Aerator.
7. At time intervals of (0 day initial) day 7, day 15, and day 30, (after application), remove 10 ml sample of water for analysis. The remaining water can be sampled at any additional time should 30 days prove inadequate for complete degradation of hydrocarbons.

NOTE: If the hydrocarbons are aged significantly, then sampling events will be changed and extended.

8. Perform EPA Test 8100 or 8030 to determine degradation.

By: Steven R. Pedigo
Chairman

SRP/AJL

OIL SPILL EATER INTERNATIONAL, CORP.

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admiralgeorge@juno.com

OIL SPILL EATER II
February 12, 2001
LIGHT-END HYDROCARBONS ON SOIL

Bioremediation Test Procedure for:
Diesel, Jet Fuel and Gasoline on Soil

Materials needed:

1. One (1) cubic Foot of Soil.
2. OSE II
3. Pan with dimensions as follows: 2 feet x 1 foot x 6 inches.
4. Natural, fresh or ocean water.
5. Light-end Hydrocarbon.

Procedure:

1. Make a solution containing 2.0 ounces of OSE II in 128 ounces (one gallon) of water. This becomes your OSE II solution. Use natural or fresh water for inland settings and natural ocean water for shoreline type cleanup or ocean settings.

2. Add 100 ml of Light-end Hydrocarbon to the soil and mix well. Spread the contaminated soil to a depth of six inches in the pan.

3. Remove 100g of soil from the pan. This soil will be analyzed as indicated below and will provide initial contamination levels to reference and compare to all other results.

4. Thoroughly wet the soil with 60 ounces of water (either fresh water or ocean water) depending on your test.

5. Since the spill quantity of light-end hydrocarbon is known (100 ml) add 100ml of the OSE II solution to the pan of contaminated soil. This application will provide one (1) part OSE II and 100 parts water to 100 parts Light-end Hydrocarbon that is recommended in OSEI literature.

6. Maintain a 30% moisture level in the soil.

7. Mix the soil by hand two times per week to allow adequate aeration and to promote bacteria motility.

8. At time intervals of initial, 3 days, 7 days and 15 days after OSE II application, remove a 100g sample of soil for analysis. The remaining soil can be sampled at any additional time should 15 days prove inadequate for complete degradation of hydrocarbons.

9. Perform EPA Tests 8015 or 8020 to determine degradation.

By: Steven R. Pedigo
Chairman

SRP/AJL

OIL SPILL EATER INTERNATIONAL, CORP.

13127 Chandler Drive
Dallas, Texas 75243
(972) 669-3390
(972) 644-8359 Fax
admiralgeorge@juno.com

OIL SPILL EATER II
February 8, 2001
TESTING - HEAVY-END HYDROCARBONS ON SOIL

Bioremediation Test Procedures for:
Crude Oil, Hydraulic Fluid, Motor Oil, Radiator Fluid,
Chlorinated Hydrocarbons, PCB'S, etc.

Materials Needed:

1. One Cubic Foot of Soil.
2. One Half Pint OSE II Concentrate.
3. Pan or Tray - 2 feet x 1 foot x 6" deep. (2' x 1' x 6" deep).
4. Natural, fresh or ocean water.
5. Heavy-end Hydrocarbons.

Procedure:

1. Make a solution containing 3 ounces of OSE II Concentrate in 128 ounces (one gallon) of water. This becomes the OSE II Solution.
2. Add 100 ml of the hydrocarbon to be tested to the soil and mix thoroughly. Spread the contaminated soil to a depth of six inches in the pan.
3. Remove 100 grams of soil from the pan. This soil will be analyzed as indicated below and will provide initial contamination levels to reference and compare to all other results.
4. Thoroughly wet the soil with 60 ounces of water, either fresh water or ocean water.
5. Since the spill quantity of the heavy- end hydrocarbon is known (100 ml), add 100 ml of the OSE II Solution to the pan of contaminated soil. This application will provide 2 parts OSE II Concentrate to 100 parts water to 100 parts of contaminated soil as recommended in OSE literature.
6. Maintain a 30% moisture level in the soil.
7. Mix with soil by hand two times per week to allow adequate aeration and to allow bacteria motility.

8. At time intervals of 0 days (initial) 3 days, 7 days, 15 days and 30 days after OSE II application, remove a 100 gram sample of soil for analysis. The remaining soil can be sampled at any additional time should 30 days prove inadequate for complete degradation of Hydrocarbons.

9. Perform EPA Tests 8100 or 8030 to determine degradation.

By: Steven R. Pedigo
Chairman

SRP/AJL

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CLEANUP PROCEDURES

OIL SPILL EATER INTERNATIONAL, CORP.

13127 Chandler Drive
Dallas, Texas 75243
(972) 669-3390
(972) 644-8359 Fax
admirallively@msn.com

April 30, 2002

OIL SPILL EATER II
PROCEDURE FOR OIL SPILL CLEANUP

GENERAL INFORMATION

It takes approximately 2 to 24 hours for OIL SPILL EATER II to penetrate the molecular wall of fresh crude oil. It takes OIL SPILL EATER II approximately 3 to 15 minutes to penetrate the molecular wall of light end petroleum or gasoline.

However, once you spray OIL SPILL EATER II on the oil, it attaches itself and will eventually engulf the oil regardless of where the oil or light petroleum may spread on ocean waters or on rivers and streams.

Additionally, once sprayed with OIL SPILL EATER II, the oil cannot attach itself to the shoreline, to rocks or to any equipment in its path.

If OIL SPILL EATER II is to be used on ocean spills or on Intertidal Zones, mix product with ocean water.

If OIL SPILL EATER II is to be used on lakes, rivers, streams, ponds or on land, mix with water from a lake, river, stream or pond.

If you are preforming a cleanup, MAKE SURE that the water used to mix with OSEII and the water used to keep area saturated is the type of water normally associated with that area. If you use fresh water it an area normally contacted with salt water or vice versa, these are different types of bacteria and competition could occur. Competition will slow the bioremediation until the area re-stabilizes.

NOTE: ***Never mix tap (faucet) water and OIL SPILL EATER II (IF POSSIBLE). The chlorine in the tap (faucet) water slows bacterial enhancement.***

These *Procedures and Application Instructions* cover Heavy End and Light End Hydrocarbons. The OSEI Corporation defines Light End Hydrocarbons as: BETX, gasoline and light solvents. Heavy End Hydrocarbons are crude oil, halogenated hydrocarbons, heavy

OIL SPILL EATER INTERNATIONAL, CORP.

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OIL SPILL EATER II (OSE II)

PROCEDURE FOR CLEANUP OF HEAVY END HYDROCARBONS - ON WATER

1. To determine quantity of *Oil Spill Eater II* concentrate needed:

A. On a Spill:

1. Use one (1) gallon of OSE II concentrate for every fifty (50) gallons of oil.

2. Use one (1) barrel of OSE II concentrate for every 2,750 gallons of oil.

B. If you know how many gallons of oil:

Multiply Gallons of oil (A) x .02 = OSE II concentrate needed

-Or-

If you know how many barrels of oil:

Multiply Barrels of oil (A) x .015 = Barrels of OSE II concentrate needed

C. If you do not know how many gallons or barrels of oil:

Multiply:	A ( ) Yds Length of Oil Slick	x	B ( ) Yds Width of Oil Slick	x	C ( ) Inches Thickness of Oil

x (.0023) = Barrels of OSE II Concentrate Needed

-Or-

x (.12) = Gallons of OSE II Concentrate Needed

II. Application Procedure:

A. Water temperature above 40° F

1. Dilute each gallon of OSE II concentrate with fifty gallons of fresh or sea water - depending on the area that is contaminated.

2. Using a helicopter or a barge with spray booms, eductor system or hand sprayer, spray the mixed OSE II onto the perimeter of the oil spill and work toward the center.

3. Next - spray OSE II over the entire surface of the spill. If the oil spill is very heavy (more than two or three inches deep), you may have to reapply OSE II to gain the one (1) part mixed OSE II to one (1) part heavy end hydrocarbon.

B. Water temperature lower than 40° F

1. Cold water reduces the rate at which OSE II enhances biodegradation of crude oil. However, biodegradation will continue to 28° F in salt water and 32.5° F in fresh water.

III. If Testing is Required:

A. Items needed:

1. An extraction device that will hold 100 ml or 3 ounces of liquid and can be pushed 6 inches or 60 cm below the water's surface.

2. 20 brown 100 ml bottles with teflon sealed caps.

3. Ice chest and ice to transport samples to the lab.

B. Pre OSE II Application Procedures:

1. Keep a daily log of observations.

2. Decide on 3 areas of the spill forming a triangle (△) to extract 3 samples.

3. Extract the 3 samples with the extraction device, pushing the collection vessel just under the surface.

4. Place each extraction in a brown jar and seal with teflon cap.

5. Mark jars (*Initial Untreated Samples*).

6. Place samples in the ice chest.

C. Perform the same steps above except pull 1 sample proximal to the spill but from an area not contaminated, affected, or impacted in any way by the spill. This is to determine what the background level or pre spill conditions are. Note the time and date of extraction.

D. 10 minutes after applying OSE II, perform the next extractions.

1. If possible, using the same triangle extraction points, push extraction device approximately 2 to 3 inches below the surface and pull extraction.

2. Decant extracted sample into a brown jar and mark initial sample 3 minutes after applying OSE II, and note the time and date of extraction.

3. Place brown jar samples in the ice chest and transport to the lab.

E. Sampling Times

1. Using procedures in D above, extract samples on day 7, day 15, day 30 and every 15 days thereafter until the acceptable level of cleanup is accomplished. Obviously, testing should cease once the acceptable levels are met.

2. In most cases, within 30 days the acceptable levels will have been accomplished.

F. Lab Tests

1. If the spill is light end hydrocarbons, then either EPA method 8015 or 8030 should be performed.

2. If the spill is heavy end hydrocarbons, then either EPA method 8030 or 8100 should be utilized.

IV. If Toxicity Testing is required:

A. Items Needed

1. An extraction device that will be capable of extracting 100 ml samples 3 meters or 3 feet below the waters' surface.

2. 12 - 100 ml brown jars with teflon seals.

3. Ice chest with ice.

B. Using instructions for extractions and the extraction time / date in III above to perform sampling

1. The 3 samples, once at the lab, should be homogenized and used for a toxicity test.

Note: In the ocean mysids, or mummichogs are generally acceptable species, and in fresh water minnows or rainbow trout are generally acceptable species.

In most cases, one toxicity test just after application of OSE II is required. However, if toxicity sampling is carried out each time efficacy testing is performed, then toxicity reduction will be proven as well.

Note: If spill is on the ocean, use ocean water to mix "OSE II." If spill is on a lake, river, stream or pond, use lake, river, stream or pond water to mix with "OSE II." To mix ocean water with anything other than ocean water and vice versa may cause adverse competition.

N E V E R mix "Oil Spill Eater II" with tap water - if possible!

PROCEDURE FOR CLEANUP OF HEAVY END HYDROCARBONS - ON WATER

OIL SPILL EATER INTERNATIONAL, CORP.

13127 Chandler Drive
Dallas, Texas 75243
(972) 669-3390
(972) 644-8359 Fax
admirallively@msn.com

OIL SPILL EATER II

PROCEDURE FOR CLEANUP OF LIGHT END HYDROCARBONS - ON WATER

1. To determine quantity of *Oil Spill Eater II* concentrate needed:

A. On a Spill:

1. One (1) gallon of OSE II concentrate for every one hundred (100) gallons of light end hydrocarbons.

2. One (1) barrel of OSE II concentrate for every 5,500 gallons of light end hydrocarbons.

B. If you know how many gallons of light end hydrocarbons spilled:

Multiply Gallons of spill (A) x .01 = Gallons of OSE II concentrate needed

-Or-

If you know how many barrels of light end hydrocarbons spilled:

Multiply Barrels of spill (A) x .0075 = Barrels of OSE II concentrate needed

C. If you do not know how many gallons or barrels of light end hydrocarbons:

Multiply: A ( ) Yds (Length of Spill) x B ( ) Yds (Width of Spill) x C ( ) Inches (Depth of Spill) x

(.0012) = Barrels of OSE II concentrate needed
(.06) = Gallons of OSE II concentrate needed

II. Application Procedure:

A. Water temperature above 40° F

1. Dilute each gallon of OSE II concentrate with one hundred gallons of fresh or sea water. Do not use fresh water on ocean water or vice versa or adverse competition may occur.

2. Using a helicopter or a barge with spray booms, eductor system set at 1%, or any spray system, spray a heavy coat of Oil Spill Eater II on the outside edges of the spill and work toward the center, if possible. This will help keep the spill from spreading.

   As the spray reaches and saturates the light end hydrocarbon molecules, emulsion will start immediately and the fire hazard will be eliminated as quickly as complete emulsion takes place. The light end hydrocarbons will eventually be converted to CO_2 and water.

3. The fire hazard should be eliminated in 4 hours or less, and the hydrocarbons should be eliminated expeditiously also.

B. Water temperature <u>below</u> 40° F

1. Cold water reduces the rate at which OSE II enhances biodegradation of hydrocarbons. However, biodegradation will continue on salt water down to 28° F, and on fresh water down to 32.5° F.

III. If Testing is Required:

A. Items needed:

1. An extraction device that will hold 100 ml or 3 ounces of liquid and can be pushed 6 inches or 60 cm below the water's surface.

2. 20 brown 100 ml bottles with teflon sealed caps.

3. Ice chest and ice to transport samples to the lab.

B. Pre OSE II Application Procedures:

1. Keep a daily log of observations.

2. Decide on 3 areas of the spill forming a triangle (△) to extract 3 samples.

3. Extract the 3 samples with the extraction device, pushing the collection vessel just under the surface.

4. Place each extraction in a brown jar and seal with teflon cap.

5. Mark jars (*Initial Untreated Samples*).

6. Place samples in the ice chest.

C. Perform the same steps above except pull 1 sample proximal to the spill but from an area not contaminated, affected, or impacted in any way by the spill. This is to determine what the background level or pre spill conditions are. Note the time and date of extraction.

D. 10 minutes after applying OSE II, perform the next extractions.

1. If possible, using the same triangle extraction points, push extraction device approximately 2 to 3 inches below the surface and pull extraction.

2. Decant extracted sample into a brown jar and mark initial sample 3 minutes after applying OSE II, and note the time and date of extraction.

3. Place brown jar samples in the ice chest and transport to the lab.

E. Sampling Times

1. Using procedures in D above, extract samples on day 7, day 15, day 30 and every 15 days thereafter until the acceptable level of cleanup is accomplished. Obviously, testing should cease once the acceptable levels are met.

2. In most cases, within 30 days the acceptable levels will have been accomplished.

F. Lab Tests

1. If the spill is light end hydrocarbons, then either EPA method 8015 or 8030 should be performed.

2. If the spill is heavy end hydrocarbons, then either EPA method 8030 or 8100 should be utilized.

Note: If spill is on the ocean, mix "OSE II" with ocean water. If spill is on a lake, river, stream or pond, mix "OSE II" with lake, river, stream or pond water.

N E V E R mix "Oil Spill Eater II" with tap water!

PROCEDURE FOR CLEANUP OF LIGHT END HYDROCARBONS - ON WATER

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PROCEDURE FOR REMOVAL OF A HYDROCARBON SHEEN ON WATER, CONCRETE, AND ASPHALT

1. To determine quantity of *Oil Spill Eater II* concentrate needed:

Multiply: A ( ) Ft. (Length of Spill) x B ( ) Ft. (Width of Spill) x .0004

= Gallons of OSE II concentrate needed

II. Application Procedure:

1. Dilute each gallon of OSE II concentrate with 50 gallons of fresh or sea water. Do not use ocean water with fresh water or vice versa because adverse competition may occur.

2. Using a barge with spray booms, hand sprayer or eductor system set at 2%, (depending on size of sheen), spray a good coating of OSE II over the entire sheen. As soon as the OSE II reaches the sheen, emulsion and solubilization will start immediately and finally conversion to CO_2 and water.

3. The hydrocarbons should be emulsified and solubilized rapidly and any fire hazards will be eliminated rapidly. Conversion to CO_2 and water is expeditious.

NOTE: If sheen is on ocean water, mix "OSE II" with ocean water. If sheen is on a lake, river, stream or pond, mix "OSE II" with lake, river, stream or pond water.

NEVER mix OSE II with tap water if possible!

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OIL SPILL EATER II

PROCEDURE FOR CLEANUP OF HYDROCARBONS - ON INTERTIDAL ZONES

1. To determine quantity of *Oil Spill Eater II* concentrate needed:

Multiply: A ( ) Yds (Length of Oil Slick) x B ( ) Yds (Width of Oil Slick) x C ( ) Inches (Thickness of Oil) x .12

= Gallons of OSE II concentrate needed

II. Application:

A. Dilute each gallon of OSE II needed (from I above) with 50 gallons of ocean water or fresh water, or mix 50 gallons of fresh or sea water, depending on area to be cleaned, with 1 gallon of OSE II. Do not use ocean water with fresh water or vice versa because adverse competition may occur.

B. It is important that you apply enough OSE II mixed 50 to 1 to get 1 part mixed OSE II to 1 part spilled hydrocarbon to ensure mobilization of oil will occur.

C. In an Intertidal Zone, it may be difficult to obtain the exact application rate, so additional applications may be necessary.

D. If necessary, Oil Spill Eater II should be applied every 48 hours in water above 40° F and every 72 hours in water below 40° F. Application should continue until oil is completely mobilized from beach area.

E. If subsurface oil occurs, OSE II will percolate along with the oil and once natural bacteria growth is started, the bacteria with its affinity for hydrocarbons, will follow the food source.

NOTE: If Intertidal Zone is in an ocean setting, mix "OSE II" concentrate with ocean water. If Intertidal Zone is a fresh water setting such as a lake, river, stream or pond, mix "OSE II" with lake, river, stream or pond water.

NEVER mix OSE II with tap water if possible!

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PROCEDURE FOR CLEANUP OF HEAVY END HYDROCARBONS - ON LAND SURFACE

Surface Spills on Land

1. To determine quantity of *Oil Spill Eater II* concentrate needed:

A. On a Spill:

1. Use one (1) gallon of OSE II for every fifty (50) gallons of oil contamination.

2. Use one (1) barrel of OSE II for every 2,750 gallons of oil contamination.

B. If you know gallons of oil contamination:

Multiply Gallons of oil contamination (A) x .02
= Gallons of OSE II concentrate needed

C. If you know barrels of oil contamination:

Multiply Barrels of oil contamination (A) x .015
= Barrels of OSE II concentrate needed

D. If you do not know gallons or barrels of oil contamination:

Multiply: A ( ) Ft. x B ( ) Ft. x C ( ) Inches x (.0125)

= Gallons of OSE II Concentrate Needed

Example: Oil spill is 120 ft. x 60 ft. and 1" thick

Multiply: 120' x 60' x 1" x .0125 = 90 gal. OSE II

E. Once the oil has seeped into the soil, then determine cubic yards of contaminated soil:

To determine Cubic Yards:

L (Ft.) x W (Ft.) x Depth (Ft.) x .037 = (B)

To determine Gallons of OSE II needed for cleanup:

Yd^3 (B) x .44 = Gallons of OSE II needed

II. Procedure:

A. Determine logistics and equipment for the particular situation. (Sample jars, mixing tank, application method, tiller, water source etc.).

B. Mix the required gallons of OSE II at a ratio of 50 gallons of water for every gallon of OSE II required.

Note: If contamination area is in contact with ocean water or spray, then use ocean water; if not, then use fresh water from the area associated with the spill. Do not add ocean water to an area not associated with ocean water or vice versa with fresh water or an adverse competition may occur among indigenous bacteria.

III. Testing:

A. Determine a grid formation for spill area.

B. Take a 50 gram extraction from each grid. Mix in a plastic bag and shake to form a composite; then perform EPA 8030 or 8100 TPH test to determine the initial TPH - and note.

C. Apply product.

D. On day 7, day 15, day 30, and every 15 days after until an acceptable TPH level is obtained, take a 50 gram extraction from each treated grid. Mix in a plastic bag to form a composite and perform EPA 8030 or 8100 TPH test to determine the extent of bioremediation. Testing should cease once the acceptable level of TPH reduction is obtained.

IV. Application:

A. Mix the required OSE II at a ratio of 50 to 1.

B. Apply the entire amount of mixed OSE II to the contamination as evenly as possible.

C. Maintain a 30% moisture level within the contamination to ensure motility and O_2.

D. If the contamination is on soil and the soil absorbs the contamination, then disc the area once a week and maintain a moisture level of 30%.

E. To determine the number of gallons of water to apply per application to maintain a 30% moisture level, take the number of gallons used to mix with OSE II concentrate and apply each time moisture content drops below 30%, and apply enough water to get the moisture level to 30%.

Note: For oil with a TPH of 100,000 and is very weathered, then additional applications of OSE II may be required.

F. When average temperature remains below 40° F during daylight hours, keep contaminated area covered with a thin translucent plastic. Continually maintain the 30% moisture level.

Note: Unless harsh winter weather persists, the plastic will help hold in the heat from the earth.

PLEASE NOTE:
The more OSE II used, the faster biodegradation will occur - up to a point. Oxygen needed for bioremediation is carried in the water and is helped by discing.

OSE II will eliminate oil spills from adding toxins to underground water systems. OSE II causes hydrocarbons to float on the surface.

These instructions are general to encompass as many situations as possible. Any specific situations should be referred to OSEI Corporation before application.

NEVER mix Oil Spill Eater II with tap water - if possible!

V. OSEI Corporation will help determine and write complete step-by-step instructions for a cleanup if you present OSEI Corporation with the complete parameters associated with a site.

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PROCEDURE FOR CLEANUP OF LIGHT END HYDROCARBONS - FOR SURFACE SPILLS ON LAND

1. To determine quantity of *Oil Spill Eater II* concentrate needed:

A. On a Spill:

1. Use one (1) gallon of OSE II concentrate for every one hundred (100) gallons of light end hydrocarbons or gasoline.

2. Use one (1) barrel of OSE II concentrate for every 5,500 gallons of light petroleum or gasoline light end hydrocarbons or gasoline.

B. If you know gallons of light end hydrocarbons or gasoline spilled:

Multiply Gallons of contaminate (A) x .01 = Gallons of OSE II needed

C. Once light end hydrocarbons or gasoline has seeped into the soil, then determine cubic yards of contaminated soil.

To determine cubic yards:

L (Ft.) x W (Ft.) x Depth (Ft.) x .037 = A (Yd^3)

To determine gallons of OSE II needed for cleanup

Yd^3 (A) x .22 = Gallons of OSE II needed

Note: Once OSE II has been applied to the soil, the fire hazard will start diminishing.

II. Procedure:

A. Determine logistics and equipment for the particular situation. (Sample jars, mixing tank, application method, tiller, water source etc.).

B. Mix the required gallons of OSE II at a ratio of 100 gallons of water for every gallon of OSE II required.

Note: If contamination area is in contact with ocean water or spray, then use ocean water from the area associated with the spill. Do not add ocean water to an area not associated with ocean water or vice versa with fresh water or an adverse competition may occur among indigenous bacteria.

III. Testing:

A. Determine a grid formation for spill area.

B. Take a 50 gram extraction from each grid. Mix in a plastic bag to form a composite. Then have a laboratory perform an EPA 8015 or 8020 TPH test to determine the initial TPH.

C. Apply product.

D. On day 7, day 15, day 30, and every 15 days thereafter until the TPH reaches an acceptable level, take a 50 gram extraction from each treated grid. Mix in a plastic bag to form a composite and have a laboratory perform an EPA 8015 or 8020 TPH test to determine the extent of bioremediation. Testing should cease once the acceptable level of TPH reduction is obtained.

IV. Application:

A. Mix the required OSE II at a ratio of 100 to 1.

B. Apply the entire amount of mixed OSE II as evenly as possible to the contamination.

C. Maintain a 30% moisture level within the contamination to ensure motility and O_2.

D. If the contamination is on soil and the soil absorbs the contamination, then disc the area once a week and maintain a moisture level of 30%.

E. To determine the number of gallons to apply per application to maintain a 30% moisture level, take the number of gallons used to mix the OSE II concentrate and apply each time moisture level drops below 30%. Apply enough of the water to get the moisture level to 30% or above.

Note: If light end hydrocarbon is weathered and aged, then additional applications of OSE II may be needed, or additional time for mitigation may be required.

F. When average temperature remains below 40° F during mitigation time, keep contaminated area covered with a thin translucent plastic and maintain the 30% moisture level with water.

Note: Unless harsh winter weather persists, the plastic will help hold in the heat from the earth.

PLEASE NOTE:

The more OSE II used, the faster the bioremediation will occur - up to a point. Oxygen needed for bioremediation is carried in the water and is helped by discing. OSE II will eliminate light end hydrocarbons spills from adding toxins to underground water systems.

These instructions are general to encompass as many situations as possible. Any special situations should be referred to OSEI Corporation before application.

NEVER mix Oil Spill Eater II with tap water, if possible!

V. **OSEI Corporation** will help determine and write complete step-by-step instructions for a cleanup if you present OSEI Corporation with the complete parameters associated with a site.

PROCEDURE FOR CLEANUP OF LIGHT END HYDROCARBONS - ON LAND

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PROCEDURE FOR CLEANUP OF HEAVY END HYDROCARBONS - FROM AN EXCAVATED SITE

1. To determine quantity of *Oil Spill Eater II* concentrate needed:

A. If you know the number of contaminated yards:

Multiply: Number of Yd^3 (A) x (.44)
= Total Gallons of OSE II needed for oil cleanup

B. If you do not know yards of contaminated soil:

Multiply:	L in Ft.	x	W in Ft.	x	D in Ft.	x	.037	=	A(Yd^3)
	Length in Feet		Width in Feet		Depth in Feet				

Use formula in A above to determine number of gallons of "*Oil Spill Eater II*" concentrate required.

II. Procedure:

A. Determine logistics, equipment and site to spread contaminated soil for the particular situation.

B. If the particular governmental regulating body requires, lay a plastic barrier in place.

C. Place contaminated soil in 24" lifts or less on the plastic barrier.

III. Application:

A. Mix the required OSE II at a ratio of 50 to 1 for the oil.

B. Apply the entire amount of mixed OSE II as evenly as possible to the contaminated soil.

C. Maintain a 30% moisture level within the contaminated soil to ensure motility and O_2.

D. To determine the number of gallons of water to apply per application to maintain a 30% moisture level, take the number of gallons used to mix with the OSE II concentrate and apply each time the moisture level drops below 30%.

E. Disc soil once a week.

Note: If contaminated soil is weather and aged, then additional application of OSE II may be needed, or additional time for mitigation may be required.

F. When temperature remains below 40° during the cleanup, keep contaminated soil covered with a thin translucent plastic and maintain a 30% moisture level.

PLEASE NOTE:
The more OSE II used, the faster bioremediation will occur - up to a point. Oxygen needed for bioremediation is carried in the water and is helped by discing. OSE II will eliminate contaminated soil from adding toxins to underground water systems. These instructions are general to encompass as many situations as possible. Any special situations should be referred to OSEI Corporation before application.

NEVER mix OSE II with tap water - if possible!

IV. Testing:

A. Determine a grid formation for contaminated soil once in place to be treated.

B. Take a 50 gram extraction from each grid and mix in a plastic bag to form a composite. Then have a laboratory perform EPA 8030 or 8100 TPH test to determine the initial TPH.

C. Apply OSE II.

D. On day 7, day 15, day 30 and every 15 days thereafter until the acceptable TPH level is obtained, take a 50 gram extraction from each treated grid. Mix in a plastic bag to form a composite and perform EPA 8030 or 8100 TPH test to determine the extent of bioremediation. Testing should cease once the acceptable level of TPH reduction is obtained.

V. **OSEI Corporation** will help determine and write complete step-by-step instructions for a cleanup if you present OSEI Corporation with the complete parameters associated with a site.

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PROCEDURE FOR CLEANUP OF LIGHT END HYDROCARBONS - FROM AN EXCAVATED SITE

1. To determine quantity of *Oil Spill Eater II* needed:

A. If you know the number of contaminated yards:

Multiply: Number of Yd^3 (A) x (.22)
= Total Gallons of OSE II needed

B. If you do not know the yards of contaminated soil:

Multiply:	L in Ft.	x	W in Ft.	x	D in Ft.	x	.037	=	A(Yd^3)
	Length in Feet		Width in Feet		Depth in Feet				

Use formula in A above to determine number of gallons of "*Oil Spill Eater II*" concentrate required.

II. Procedure:

A. Determine logistics, equipment and site to spread contaminated soil for the particular situation.

B. If the particular governmental regulating body requires, lay a plastic barrier in place.

C. Place contaminated soil in 24" lifts or less on the plastic barrier.

III. Application:

A. Mix the required OSE II at a ratio of 100 to 1 for light end hydrocarbons.

B. Apply the entire amount of mixed OSE II as evenly as possible to the contaminated soil.

C. Maintain a 30% moisture level within the contaminated soil to ensure motility and O_2.

D. To determine the number of gallons of water possible to apply per application to maintain a 30% moisture level, take the number of gallons used to mix with the OSE II concentrate and apply each time the moisture level drops below 30%.

E. Disc soil once a week.

Note: If contaminated soil is weather and aged, then additional application of OSE II may be needed, or additional time for mitigation may be required.

F. When temperature remains below 40° F during the cleanup, keep contaminated soil covered with a thin translucent plastic, and maintain a 30% moisture level.

IV. Testing:

A. Determine a grid formation for contaminated soil once in place to be treated.

B. Take a 50 gram extraction from each grid. Mix in a plastic bag to form a composite; then perform EPA 8015 or 8020 method TPH test to determine the initial TPH - and note.

C. Apply product.

D. On day 7, day 15, day 30 and every 15 days thereafter until the acceptable TPH level is obtained, take a 50 gram extraction from each grid and place in a plastic bag. Mix it to form a composite. Perform EPA 8015 or 8020 method test to determine TPH level. Testing should cease once the acceptable level of TPH reduction is obtained.

Note: The more OSE II used, the faster bioremediation will occur - up to a point. Oxygen needed for bioremediation is carried in the water and is helped by discing. OSE II will eliminate contaminated soil from adding toxins to the underground water systems. These instructions are general to encompass as many situations as possible. Any special instructions should be referred to OSEI Corporation before application.

NEVER mix OSE II with tap water (if possible)!

V. OSEI Corporation will help determine and write complete step-by-step instructions for a cleanup if you present OSEI Corporation with the complete parameters associated with a site.

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PROCEDURE FOR CLEANUP OF OIL SPILLS ON CONCRETE OR ASPHALT

1. **LIGHT END HYDROCARBONS:**

   a. Estimate gallons of spilled fuel.
   b. Use 1.5 ounces of OSE II concentrate per spilled gallon.
   c. Use 1 gallon of water per spilled gallon.
   d. Mix OSE II with water.
   e. Spray on spill.
   f. Allow OSE II to react for 20 minutes.
   g. Either (1) wash off with water or (2) simply allow residue to evaporate.

2. **HEAVY END HYDROCARBONS:**

   a. Follow same procedure as in 1 above, except use 3 ounces of OSE II for spilled gallons of heavy oils.
   b. If possible, use stiff brush to agitate.
   c. Allow OSE II to react for 30 minutes.
   d. Wash off with water.

3. **THICK AND OLD OIL STAINS:**

   a. Follow procedure in 1 above.
   b. Use 4 ounces of OSE II and 1 gallon of water per every 9 square feet of contaminant.
   c. Brush vigorously with stiff brush.
   d. Allow OSE II to react for 30 minutes.
   e. Wash off with water.
   f. Repeat process, if required.

NOTE: Old oil on concrete may imbed carbon into concrete. OSE II will not remove this black carbon. However, carbon is inert and non-toxic.

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PROCEDURE AND APPLICATIONS
FOR HYDROCARBON CLEANUP
UNDER BUILDINGS, IMMOVABLE OBJECTS, UNDERGROUND GROUNDWATER

OSEI Corporation will help determine and write step-by-step procedures for cleaning up these types of sites. You will have to supply OSEI Corporation with all the parameters involving your particular site.

There are so many potential variables associated with these types of cleanups, it is difficult to write general instructions that would encompass all the variables.

Please contact us at:

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13127 Chandler Drive
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George

O.A. (George) Lively
Rear Admiral (RET)
President

OAL/eem

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OIL SPILL EATER II

PROCEDURE FOR CLEANUP OF BIRDS AND MAMMALS

Dilution for application to animals or birds should be one (1) part "*Oil Spill Eater II*" concentrate to sixty (60) parts water. Feathered animals should not be released after cleaning until natural oils have been restored.

NOTE: *It is OKAY to mix with tap water; however, sterile water, ocean water or fresh water is preferable.*

Steven R. Pedigo
Chairman

SRP/eem

OIL SPILL EATER INTERNATIONAL, CORP.

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A SUCCESSFUL USER OF
OIL SPILL EATER II
ON WATER

Dear Environmental Manager:

Mr. Steve Fry at the Navy Fuel Farm, Pt. Loma, San Diego, California, has used Oil Spill Eater II for fuel spills both on land and water for over one year very effectively. Mr. Fry has reduced his cleanup cost on water spills from $90.00 per gallon to $12.00 per gallon using OSEII and only $1.00 of the $12.00 is the cost of OSEII.

We hope you will try Oil Spill Eater II. It works and is effective!

Sincerely,

O. A. (George) Lively
Real Admiral (RET)
President

OAL/MFK

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SUBJECT: Volunteer Groups (Homer Alaska)

SITE: Homer Alaska Beach that was contaminated by the Exxon Valdez

TEST: Performed by Bill Day

9/10/89 at 8 a.m.

Volunteers mixed 2 gallons of Alaskan sea water, 1 gallon tar balls, sticks heavily oiled and put 8 ozs of Oil Spill Eater in a 5 gallon bucket and let stand stirring every 12 hours exposed to temperature highs of 50° and lows of 30°f.

9/12/89 - Observations and Conclusions

Sky Blue's Oil Spill Eater is very effective in removing oil from sediments, rocks and organics. Sticks and debris has settled to the bottom of the bucket and is clean in appearance.

9/17/89

A final observation of bucket test shows that the heavily oiled pine needles sticks have settled to the bottom of the bucket and are free of detectable oil. They are no longer sticky to the touch. The water seems to be clean. There is no visible sheen in the bucket.

OVERALL CONCLUSIONS

I highly recommend further cleaning experimentation on rocks, cliffs, driftwood and sediments.

9/18/89

2 gallons of Oil Spill Eater was mixed with approximately 100 gallons of sea water pending ADEC approval for further testing.

9/19/89

Oil Spill Eater mixture was accidentally knocked over by a rock washing machine and mixture poured out onto the beach into the intertidal zone.

9/20/89

Oiled beach where OSE poured onto is free of detectable oil on the surface and subsurface with no detectable release of sheen on the water surface.

9/21/89

Oil Spill Eater was very effective in breaking the oil down and removing it from the beach with no apparent side affects.

9/22/89

Homer Volunteer group will ask ADEC to fund their clean up of their beaches with Oil Spill Eater.

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OSMI COMPANY - GALVESTON, TEXAS

DIESEL SPILL CLEAN UP

In August 1990, a yacht discharged approximately 50 gallons of diesel Fuel into a yacht basin in Galveston Bay. The diesel Slick was spreading rapidly and the owner did not have a solution.

OSMI Management happened to be present and had some "OIL SPILL EATER" II (OSE) with them. They quickly filled several hand sprayer bottles with OSE and bay water. Using small boats they quickly encircled the Diesel Sheen (which was then approximately 300 yards in length - and by spraying OSE around the diesel spill perimeter, OSMI Management then sprayed (covered) the entire spill.

The owner was amazed to see the Diesel Sheen disappear in a matter of minutes. Of course, the sheen disappeared in minutes but the actual bioremediation of the hydrocarbons took several hours.

Conclusion: "OIL SPILL EATER " II eliminated a hydrocarbon spill (diesel fuel) effectively and vitually immediately!.

Steven R. Pedigo
Chairman

OIL SPILL EATER INTERNATIONAL, CORP.

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SUMMARY

DALLAS NAVAL AIR STATION

OIL SPILL

On January 18, 1995, due to very heavy rains, there was an overflow of 2,000 gallons of JP-4, JP-8 and motor oil behind Building #193 at NAS. The overflow went through a drain pipe on to a neighboring golf course.

NAS personnel began applying OIL SPILL EATER II (OSE) on January 19, 1995 at a 50 to 1 ratio with water, which they applied with hand held sprayers. Over a period of weeks, they applied 40 gallons of OSE and 2,000 gallons of water.

NAS personnel did not perform initial TPH sampling of the contaminated soil but knew from the amount of oil, odor and visual observation of it's severity.

The attached final soil sampling was performed in four (4) different areas using EPA methods 8020/5030 for BETX and 418.1 for total hydrocarbon count. In all four (4) sampling areas the BETX and total hydrocarbons were reduced well below state acceptance levels for contaminant soil of 100 ppm.

In addition, the grass where OSE was applied to the contaminated soil is now lush green!

O. A. Lively
Rear Admiral (ret)
President

OAL/AJL

Report #	: 95-1626 01	Date Received	: 08/29/95
Sample ID	: 10928 S-9-1	BTEX Analysis Date	: 09/05/95
Project #	: 10928	TPH Extraction Date	: 08/31/95
Sample Matrix	: Soil	TPH Analysis Date	: 08/31/95
Depth Interval	: N/A		
Analyst	: JSL		

Compound	Results	Practical Quantitation Limit
Benzene	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Toluene	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Ethylbenzene	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Total Xylenes	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Total BTEX (Calculated)	* BPQL µg/Kg (ppb)	2 µg/Kg (ppb)
Total Petroleum Hydrocarbons	43 mg/Kg (ppm)	10 mg/Kg (ppm)

*Below Practical Quantitation Limits

Method: BTEX - EPA Method 8020A/5030 - SW-846
TPH - EPA Method 418.1/3550 - SW-846

Joe Thompson
Director of Technical Services

John S. Lee
Analytical Chemist

Report #	: 95-1626-02	Date Received	: 08/29/95
Sample ID	: 10928 S-9-2	BTEX Analysis Date	: 09/05/95
Project #	: 10928	TPH Extraction Date	: 08/31/95
Sample Matrix	: Soil	TPH Analysis Date	: 08/31/95
Depth Interval	: N/A		
Analyst	: JSL		

Compound	Results	Practical Quantitation Limit
Benzene	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Toluene	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Ethylbenzene	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Total Xylenes	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Total BTEX (Calculated)	* BPQL µg/Kg (ppb)	2 µg/Kg (ppb)
Total Petroleum Hydrocarbons	96 mg/Kg (ppm)	10 mg/Kg (ppm)

*Below Practical Quantitation Limits

Method: BTEX - EPA Method 8020A/5030 - SW-846
TPH - EPA Method 418.1/3550 - SW-846

Joe Thompson
Director of Technical Services

John S. Lee
Analytical Chemist

Report #	: 95-1626-03	Date Received	: 08/29/95
Sample ID	: 10928 S-9-3	BTEX Analysis Date	: 09/05/95
Project #	: 10928	TPH Extraction Date	: 08/31/95
Sample Matrix	: Soil	TPH Analysis Date	: 08/31/95
Depth Interval	: N/A		
Analyst	: JSL		

Compound	Results	Practical Quantitation Limit
Benzene	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Toluene	3 µg/Kg (ppb)	2 µg/Kg (ppb)
Ethylbenzene	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Total Xylenes	2 µg/Kg (ppb)	2 µg/Kg (ppb)
Total BTEX (Calculated)	5 µg/Kg (ppb)	2 µg/Kg (ppb)
Total Petroleum Hydrocarbons	27 mg/Kg (ppm)	10 mg/Kg (ppm)

*Below Practical Quantitation Limits

Method: BTEX - EPA Method 8020A/5030 - SW-846
TPH - EPA Method 418.1/3550 - SW-846

Joe Thompson
Director of Technical Services

John S. Lee
Analytical Chemist

Report #	: 95-1626-04	Date Received	: 08/29/95
Sample ID	: 10928 S-9-4	BTEX Analysis Date	: 09/05/95
Project #	: 10928	TPH Extraction Date	: 08/31/95
Sample Matrix	: Soil	TPH Analysis Date	: 08/31/95
Depth Interval	: N/A		
Analyst	: JSL		

Compound	Results	Practical Quantitation Limit
Benzene	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Toluene	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Ethylbenzene	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Total Xylenes	<2 µg/Kg (ppb)	2 µg/Kg (ppb)
Total BTEX (Calculated)	* BPQL µg/Kg (ppb)	2 µg/Kg (ppb)
Total Petroleum Hydrocarbons	23 mg/Kg (ppm)	10 mg/Kg (ppm)

*Below Practical Quantitation Limits

Method: BTEX - EPA Method 8020A/5030 - SW-846
TPH - EPA Method 418.1/3550 - SW-846

Joe Thompson
Director of Technical Services

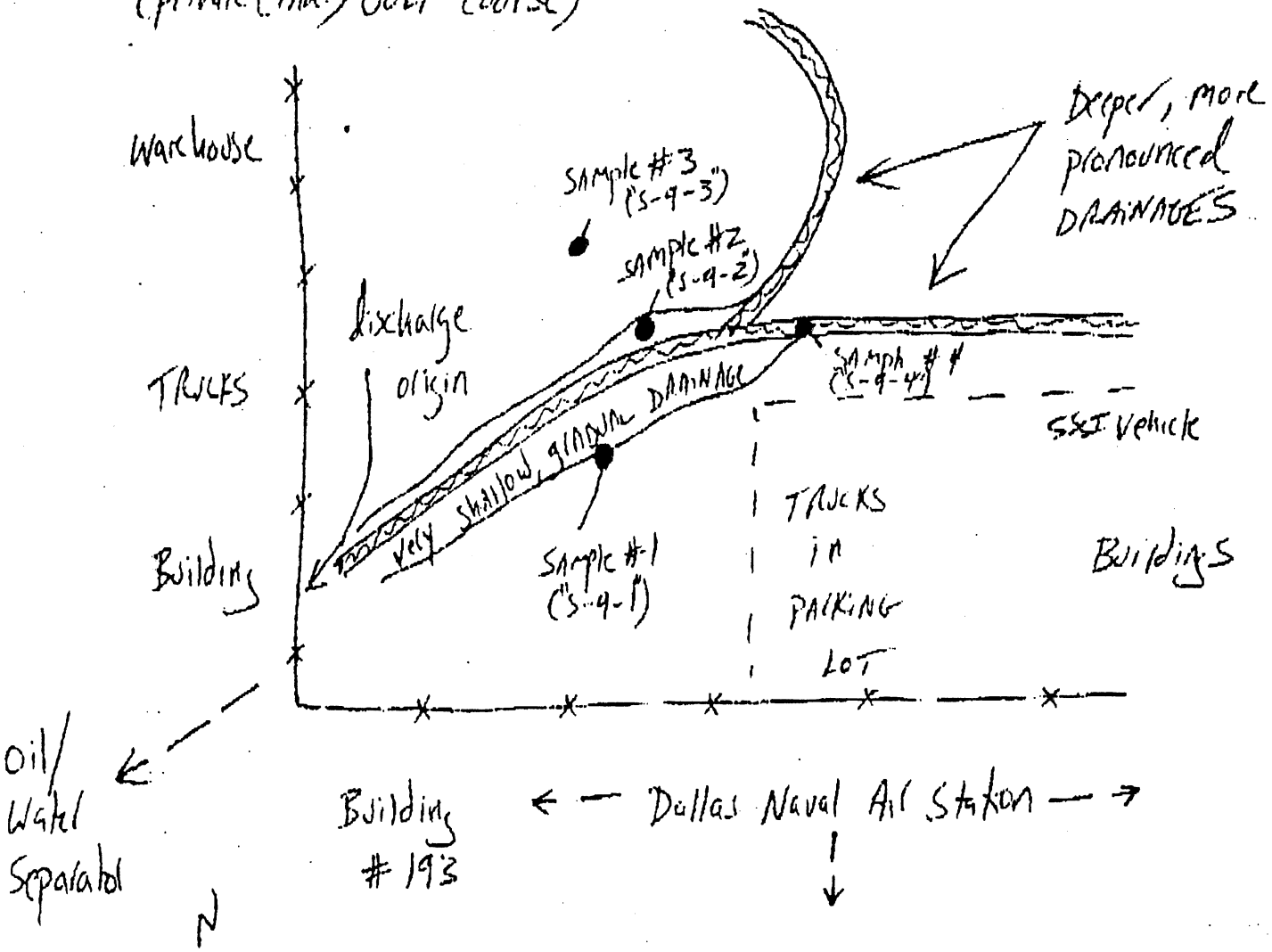
John S. Lee
Analytical Chemist

DALLAS NAVAL AIR STATION Field Notes

8/29/95
1:00 P.M.

Outfall Behind Building #193 (Due to very heavy rainfall)

(private (ind.) GOLF course)



Sample #1 - Top 4"* in edge of drain. where may have
Sample #2 - 1' to 2' deep from depression in drain
Sample #3 - Surface to 2' deep on hill away from
Sample #4 - Surface to 1.5' deep in deep, wet drai

* Construction debris used as fill (reportedly) prevented further Sampling.
Soil was a Sandy Loam w/ varying amounts of CLAY encountered.

SUCCESS STORY

24 August 1994

Lawrence M. Brennan, Jr.
3400 Forest Way Court
Arlington, TX 76017

*Mr. Brennan is a Retired Commander. He was the Environmental Manager for the Naval Air Station in Dallas, Texas.

O. A. (George) Lively
Oil Spill Eater International, Corp.
13127 Chandler Drive
Dallas, Texas 75230

Dear George:

I would like to take this opportunity to tell you how impressed I am with your "Oil Spill Eater II" (OSE II) petroleum product remediator.

Prior to my retirement from the U.S. Navy, I was the Environmental Officer at a large Reserve Naval Air Station. Our goal was to maintain Environmental Compliance and our workload was enormous. We never had to respond to a major petroleum spill but we were constantly being called to cleanup small petroleum product spills associated with aircraft maintence and lax housekeeping practices. The most important task when responding to a spill was to prevent harmful contaminants from entering the drainage systems. We needed a product to help us to these incidents; that was easy and quick to apply; and was economical. The product was OIL SPILL EATER II.

My staff and I were skeptical when you first demonstrated OSE II, but it did work and on the light petroleum products associated with the aviation industry, ie. JP-5 aviation fuel, hydraulic fluids, and lubricants, it worked extremely well. On numerous occasions when hydraulic fluids would be released on the ramp during aircraft maintenance operations, application of OSE II would remove the oily texture of the spent fluids generally within an hour and the resulting waters would soon evaporate. When a contractor spilled diesel fuel on a parking lot during equipment refueling, our responders had to act quickly in rainy weather. We first erected booms at the storm drain discharges then sprayed the spreading film with OSE II. The spill was not large but was moving fast in the wet conditions. After cleaning the area with absorbent pads and vacuum we pulled and analyzed water samples from the adjacent storm drains. The resulting TPH analysis showed only slight traces of petroleum product.

We used OSE II twice during aircraft crash responses. The most significant was the crash of a jet fighter aircraft. The aircraft was totally destroyed on impact and the ensuing fire. Much of the burning fuel ran into a nearby water holding tank. After securing the crash scene we sprayed all affected areas around and in the tank. The next day we prepared to remove any petroleum products visible but there were none. After coordinating with the regulators we took nineteen separate water samples from various locations on the pond and had complete BTEX/TPH analysis run. Half of the samples had no detectable findings while the rest showed only negligible traces of petroleum hydrocarbon.

We used OSE IIfor general housekeeping around petroleum products storage areas, for product release during underground storage tank removal and numerous response situations. The product never failed to work as advertised. When we had reason to analyze water samples that had been contaminated with petroleum product and after using your Oil Spill Eater II, the results continually showed minimal petroleum hydrocarbon residues and consistently well below regulatory levels of concern. In all response situations we first contained the spill then picked it up with vacuum and absorbent materials. OSE II was used in conjunction with these other procedures. I feel your product is an excellent solution for remediating petroleum product spills when combined with standard cleanup procedures.

I strongly feel that Oil Spill Eater II will effectively and efficiently remediate any petroleum hydrocarbon contamination situation. Time will vary depending on weight of petroleum product, consistency of median and environmental conditions. Most importantly, it is a product friendly to the environment. It transforms harmful containments to more environmentally friendly substances in our soil and water.

Very respectfully,

L.M. Brennan, Jr.

13127 Chandler Drive
Dallas, Texas 75243
(972) 669-3390
(972) 644-8359 Fax
admirallively@msn.com

WHERE OSE II HAS BEEN USED

OSE II can be used virtually anywhere that can sustain Microbial Life

Oceans

Lakes

Rivers, Streams

Where Fresh and Ocean Water come together (Brackish Water)

All Types of Soil

Rocky Areas - Pebbled Areas

Marshes - Estuaries

Underground Soil

Underground Water

Birds, Mammals, Living Creatures

Under Buildings & Immovable Objects (In-Situ)

Animal Clinics

Kitchens, Restaurants (Grease Traps)

WHO CAN USE OSE II

Manufacturing Plants that use Organic Based Natural Resources

Manufacturing Plants that use Hydrocarbon Based Natural Resources

Any Owner, Operator of Engines or Robotics

All Transportation Groups Air, Rail, Bus, Water

Utility Industry

Refineries, Oil Tankers, Drilling Companies, Pipeline Operators

Military

Governments

Fire Departments

Ports and Harbors

Homeowners that spill Fuel, Oil, Pesticides, or Solvents

Septic Tanks, Sewer Treatment Plants, Farms and Ranches

Cleanup Contractors

Insurance Companies (Insurance Adjustors)

USES OF AND CONTAMINANTS THAT OSE II CAN OR HAS BIOREMEDIATED

This list contains most of the hazardous material OSE II has bioremediated. It is not complete. We add new compounds continually. This list is to give you an idea of what OSE II can remediate. If your particular contaminant is not listed, please call us.

OSE II can Bioremediate
Most Organic Based Compounds

OSE II can Bioremediate almost
all Hydrocarbon Based Compounds

Some of the Hazardous Material
OSE II has Bioremediated

- All types of Gasoline
- Diesel Fuel
- Jet A
- JP 4
- JP 5
- JP 8
- Numerous Solvents
- Crude Oils
    - Alaskan North Slope Crude Oil
    - Texas Sweet Crude Oil
    - South African Crude Oil
    - Bunker C Crude Oil
    - Venezuelan Crude Oil
    - Mexican Crude Oil
    - Louisiana Crude Oil
    - Kuwait and Saudi Arabian Crude Oil
- Pesticides
    - DDT
    - Malathion
    - Organo Pesticides
- Other Compounds
    - Tert Butyl Ether
    - Benzene
    - Zylene
    - Toluene
    - Ethyl Benzene
    - Chrysene
    - Hopane
    - Hexadecane
    - Naphthalene
    - Fluorene
    - Phytane
    - Phenanthrene
    - C18
    - C30
    - Pristane
    - And Others
    - No 2 and No 6 Heating Oils
    - Kerosene
    - Grease form Animals
    - Grease from Vegetables
    - Dioxins
    - Furans
    - Creosote
    - PCBs (Poly Chlorinated Biphenols)
    - Dry Cleaning Fluid - (Perchloralethylene)
    - Ethylene Glycol - (Radiator Fluid)
    - Deicing Agent
    - Hydraulic Oil
    - Brake Fluid
    - Power Steering Fluid
    - Motor Oils
    - Co Polymers
    - TNT
    - Gun Powder

NRT SCIENCE AND TECHNOLOGY COMMITTEE

Fact Sheet: Bioremediation in Oil Spill Response

An information update on the use of bioremediation.

May, 2000

1. The purpose of this fact sheet is to provide on scene coordinators and other decision-makers with the latest information on evolving technologies that may be applicable for use in responding to an oil spill. Bioremediation is one technique that may be useful to remove spilled oil under certain geographic and climatic conditions. For the purpose of this effort, bioremediation is defined to include the use of nutrients to enhance the activity of indigenous organisms and/or the addition of naturally-occurring non-indigenous microorganisms. This fact sheet is an update of the NRT Science and Technology's 1991 Bioremediation fact sheet.

2. Bioremediation is a technology that offers great promise in converting the toxigenic compounds of oil to nontoxic products without further disruption to the local environment. Bioremediation is typically used as a polishing step, after conventional cleanup methods have been used. Bioremediation products considered for use during spill cleanup operations must be listed in accordance with the requirements of Subpart J of the National Contingency Plan (for further information on product listing, please consult EPA's Oil Program website at www.epa.gov/oilspill). Genetically engineered organisms are not being considered for use at this time by EPA for oil spill and are therefore not discussed in this fact sheet.

REQUIREMENTS FOR SUCCESS

3. Several factors influence the success of bioremediation, the most important being the type of bacteria present at the site, the physical and chemical characteristics of the oil, and the oil surface area. The two main approaches to oil-spill bioremediation are: (1) *bioaugmentation,* in which oil-degrading bacteria are added to supplement the existing microbial population, and (2) *biostimulation*, in which nutrients, or other growth limiting substances, are added to stimulate the growth of indigenous oil degraders.

4. Addition of oil-degrading bacteria has not been shown to have any long-term beneficial effects in shoreline cleanup operations because:

5. The size of the hydrocarbon-degrading bacterial population usually increases rapidly in response to oil contamination, and it is very difficult, if not impossible, to increase the microbial population over that which can be achieved by biostimulation alone[1-4];

6. The carrying capacity of most environments is probably determined by factors such as predation by protozoans, the oil surface area, or scouring of attached biomass by wave activity that are not affected by bioaugmentation; and

7. Added bacteria seem to compete poorly with the indigenous population.[5,6]

8. Under the appropriate conditions, biostimulation has been shown to have beneficial effects in shoreline cleanup operations. The main challenge associated with biostimulation in oil-contaminated coastal areas or tidally influenced freshwater rivers and streams is maintaining optimal nutrient concentrations in contact with the oil.

NUTRIENT APPLICATION

9. Effective bioremediation requires that (1) nutrients remain in contact with the oiled material, and (2) nutrient concentrations are sufficient to support the maximal growth rate of the oil-degrading bacteria throughout the cleanup operation.

10. Open Water Environments. Bioremediation of open water spills is not considered to be appropriate or achievable because of the above two requirements. When nutrients are added to a floating slick, they immediately disperse into the water column, essentially diluting to background levels. At such levels rapid conversion of the hydrocarbons to biomass, CO_2, and other innocuous end products would not be readily supported.

11. Marine Environments. Contamination of coastal areas by oil from offshore spills usually occurs in the intertidal zone where the washout of dissolved nutrients can be extremely rapid. In 1994 and 1995, studies were conducted on the shorelines of Delaware[7] and Maine[8] to study the rate of nutrient transport in low and high energy sandy beaches. These studies found that surface application of nutrients (including slow-release or oleophilic formulations) is ineffective on high-energy beaches because most of the nutrients are lost to dilution at high tide. However, on low

11. (continued)

energy beaches surface application of nutrients was found to be an effective and economical bioremediation strategy. Subsurface application of nutrients might be more effective on high-energy beaches but because crude oil does not penetrate deeply into most beach matrices, it is difficult to insure that the nutrients reach the oil-contaminated area near the surface.

12.
Freshwater Environments. An oil spill is most likely to have the greatest impact on wetlands or marshes. Less research has been conducted in these types of environments, so it is not yet known how well bioremediation would enhance oil removal. However, the same principles apply to this type of environment as in the marine environment: nutrients must remain in contact with the oiled material, and nutrient concentrations must be sufficient to support the maximal growth rate of the oil-degrading bacteria. There is an added complication in a wetland; oil penetration is expected to be much lower than on a porous, sandy marine beach. Below only a few centimeters of depth, the environment becomes anaerobic, and petroleum biodegradation is likely to be much slower even in the presence of an adequate supply of nitrogen and phosphorus. Technology for increasing the oxygen concentration in such an environment is still undeveloped, other than reliance on the wetland plants themselves to pump oxygen down through the root system. By the year 2000, however, data will be available from an intentional oil spill study being conducted jointly by the U.S. EPA and Fisheries and Oceans-Canada on a freshwater shoreline of the St. Lawrence River in Quebec. This study is examining bioremediation with nitrate and ammonium in the presence and absence of wetland plant species (*Scirpis americanus).*

13.
Soil Environments. Land-farming techniques have been used extensively by petroleum companies and researchers for treating oil spills on soil. Again, the same principles apply: nutrients must remain in contact with the oiled material, and nutrient concentrations must be sufficient to support the maximal growth rate of the oil-degrading bacteria. For surface contamination, maintenance of an adequate supply of oxygen is accomplished by tilling. The maximum tilling depth is limited to about 15 to 20 inches. If the contamination zone is deeper, other types of technologies are used, such as bioventing, composting, or use of biopiles, all of which require addition of an external supply of forced air aeration.

14.
FIELD EVIDENCE FOR BIOREMEDIATION

Demonstrating the effectiveness of oil spill bioremediation technologies in the field is difficult because the experimental conditions cannot be controlled as well as is in the lab. Nevertheless, well-designed field studies can provide strong evidence for the success of a particular technology if one can convincingly show that (1) oil disappears faster in treated areas than in untreated areas and (2) biodegradation is the main reason for the increased rate of disappearance. Convincing demonstration of an increased rate of oil degradation was provided from a field study conducted during the summer of 1994 on the shoreline of Delaware Bay[9]. Although substantial hydrocarbon biodegradation occurred in the untreated plots, statistically significant differences between treated and untreated plots were observed in the biodegradation rates of certain hydrocarbon compounds.

15.
To distinguish between oil lost by physical means and oil that has been biodegraded, biodegradable constituents are normalized to a resistant biomarker compound. Hopanes often serve as this biomarker compound because they are highly resistant to biodegradation and exist in all crude oils. Normalizing to hopane automatically accounts for disappearance of oil by physical washout mechanisms. In refined oils that have no hopanes biodegradation can be confirmed by normalizing to a highly substituted 4-ring PAH or by examining the relative rates of disappearance of alkanes and PAH homologs.

16.
It is important to note that some bioremediation products contain surfactants and emulsifiers that change the appearance and mobility of the oil. These processes should be distinguished from true biodegradation.

OTHER RESEARCH

17.
Research is ongoing to evaluate bioremediation and phytoremediation (plant-assisted enhancement of oil biodegradation) for their applicability to clean up oil spills contaminating salt marshes and freshwater wetlands. By December of 2000, EPA is planning to produce a draft guidance document detailing the use of bioremediation for sandy marine beaches and freshwater wetlands. EPA is also studying the biodegradability of non-petroleum oils (vegetable oils and animal fats) and their impacts on the environment during biodegradation. Reports will be available some time in 2000 and 2001.

CONCLUSION

18.
In conclusion, bioremediation is a proven alternative treatment tool that can be used in certain oil-contaminated environments. Typically, it is used as a polishing step after conventional mechanical cleanup options have been applied. It is a relatively slow process, requiring weeks to months to effect cleanup. If done properly, it can be very cost-effective, although an in-depth economic analysis has not been conducted to date.

18. (continued)
One of the advantages to using bioremediation products is that the toxic hydrocarbon compounds are destroyed rather than simply moved to another environment. The biggest challenge facing the responder is maintaining the proper conditions for maximal biodegradation to take place, i.e., maintaining sufficient nitrogen and phosphorus concentrations in the pore water at all times. Based on field experiments and solid evidence from the literature it has been shown that addition of exogenous cultures of microorganisms will not enhance the process more than simple nutrient addition and that bioremediation is less effective on high energy shorelines.

The NRT S&T Committee technical contact for bioremediation issues is Dr. Albert D. Venosa of the Environmental Protection Agency. He can be reached at venosa.albert@epa.gov.

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OIL SPILL EATER INTERNATIONAL, CORP.

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Dallas, Texas 75243
(972) 669-3390
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admiralgeorge@juno.com

OIL SPILL EATER INTERNATIONAL (OSEI, CORP.) EVALUATION OF THE NRT SCIENCE AND TECHNOLOGY COMMITTEE FACT SHEET: MAY 20, 2000

Paragraph 1. Is a Statement of the Fact Sheet's Purpose.

It is unfortunate that Dr. Venosa chose to only use nutrients for the tests performed for this Fact Sheet. We agree - nutrients alone will not work - and Dr. Venosa proves this fact in his Fact Sheet. Dr. Venosa keeps pushing nutrients which are very limited as to the spill conditions in which they may be used effectively; as Dr. Venosa points out.

Paragraph 2.

Explains that Bioremediation offers significant promise in converting the toxigenic compounds of oil to non-toxic products without further disruption to the environment. Again, Dr. Al Venosa (EPA Laboratory) keeps pushing nutrients but then proves they do not work. How does this help the On-Scene Coordinators?

Paragraph 3. Requirements for Success.

They describe Biostimulation as nutrients or other growth-limiting substances, but they fail to mention or test those Bioremediation Products that utilize nutrients all the other constituents to emulate Mother Nature.

Paragraphs 4 through 7.

We agree with the EPA Fact Sheet. For eleven years we have stated that using indigenous bacteria to clean up oil spills works faster and more effective than adding bacterial product.

Paragraph 8.

They explain that under the appropriate conditions, biostimulation has been shown to have beneficial effects on shoreline treatments. This statement needs to be qualified as nutrients only (which Dr. Venosa keeps pursuing) are limited as to the conditions in which they may be used.

OIL SPILL EATER II is not limited the way nutrients are. In fact, in a letter dated April 20, 2000, Mr. Venosa agreed to the fact that when OSE II is applied to oil, it adheres to the oil. This means wave action will not wash away OSE II and dilute it. This means OSE II can be used in active inter-tidal zones, as well as open ocean settings and fresh water fast moving rivers.

Paragraph 9. Nutrient Application.

OSEI, Corp. concurs with this paragraph since OSE II does exactly what Dr. Venosa states is necessary for "effective Bioremediation." OSE II (1) adheres to the oil and (2) supplies the concentration of all nutrients necessary for effective Bioremediation.

Paragraph 10. Open Water Environments.

They state that Bioremediation of open waters is not considered appropriate or achievable. What Dr. Venosa is really stating is that what nutrients alone are limited as to where they can be used. This is not true for OIL SPILL EATER II (OSE II), since it molecularly adheres to the oil and Dr. Venosa has so stated and knows that OSE II does.

How does Dr. Venosa explain and ignore the fact that for one and one/half years OSE II has been successfully and effectively used at the Navy Fuel Farm in San Diego, CA for oil spills on U.S. Navigable Waters, with the Coast Guard and the State of California present? The oil is cleaned up and with no adverse effects to the San Diego Bay ECO System.

Furthermore, Dr. Venosa has been fully appraised of these facts. He obviously is choosing to ignore the fact that at least one Bioremediation Product does work effectively on water. Dr. Venosa needs to change this statement in the Fact Sheet since he has misled the NRT', the RRT's and particularly the OSC's.

Paragraph 11. Marine environments.

OSEI, Corp. concurs with their comments, but they are only applicable to nutrients, - not OIL SPILL EATER II.

Paragraph 12. Fresh Water.

OSEI, CORP. agrees with the EPA - nutrients have limited capabilities; however, OSE II breaks up the oil in small droplets, OSE II "floats" the oil (hydraulic lifting) and OSE II molecularly adheres to the oil. OSE II will only minimally increase the BOD (See Enclosure #1 - BOD statement by Dr. Theron Miller). If the BOD becomes a problem in an enclosed environment, simply aerating the oil-covered water with pumps, will allow rapid Biodegradation of the oil and eliminate the BOD problem.

Paragraph 13. Soil Environments

Again, nutrients (fertilizers) do not adhere to the oil and, how many nutrients do you apply? OSE II has been solving this problem for 11 years. We have been cleaning up soil that is contaminated with hydrocarbons very effectively and at a tremendous savings in cost.

Paragraph 14. Field Evidence for Bioremediation.

The Fact Sheet states that it is difficult to demonstrate Bioremediation in the field vs. the lab. OSE II has cleaned up contaminated soils all over the U.S., Alaska, Korea and Japan.

Using Dr.Venosa's nutrients, it is impossible to demonstrate for the reasons mentioned previously, i.e., nutrients do not adhere to the oil; how much product (nutrients) do you use; and Dr.Venosa's nutrients do not contain all the nutrients necessary for the complete bacterial growth. OSE II provides all the nutrients needed and can tell the user exactly how much OSE II to apply.

Paragraph 15.

OSEI, Corp. has proven that OSE II does, in fact, biodegrade oil. Dr. Brown of the University of Alaska, ran a scientifically valid test to prove that OSE II does biodegrade alkanes and PAH's. Dr. Venosa has this test and is fully aware that OSE II works whereas his nutrients will not. (See Enclosure 2, a copy of Dr. Brown's Test.).

Paragraph 16. BIOREMEDIATION - WHAT IT REALLY IS!

OIL SPILL EATER II
CHEMICAL PROCESS

Once OSE II is applied to a hydrocarbon spill, the enzymes and other product constituents start emulsification and solubalization of the hydrocarbon substrate. Emulsification and solubalization generally take from a few minutes up to a few hours for heavy-end hydrocarons, once OSE II is applied, with a Temperarture of 40 degrees F. or greater. Once solubilization is completed, the hydrocarbon substrate is less toxic (and the hazard of a fire is diminished) the enhanced, naturally occurring bacteria will have a higher affinity for the solubilized, hydrocarbon substrate.

NOTE: There is no hydraulic loading with the use of OSE II and therefore treated hydrocarbons are not pushed into the lower depths of the water column. During these reactions, OSE II offers up a complete nutrient system to promote the rapid growth or colonization of naturally occurring, indigenous bacteria.

OSE II is also formulated so that once application to the hydrocarbon substrate occurs, molecular adhesion takes place. This prevents OSE II from being removed from the hydrocarbons easily. The above reaction forms the substrate complex.

Once the outer molecular walls of the hydrocarbon substrate complex have been weakened or broken, then this allows bacteria better access to the hydrocarbon substrate. The nutrients in OSE II's product matracies (readily available nitrogen, phosphorous, carbon and vitamins), rapidly populates naturally occurring bacteria. There are certain product constituents to enhance various hydrocarbon- degrading bacteria specifically. The naturally enhanced hydrocarbon degrading bacteria rapidly populate until product nutrients are depleted, at which time they readily convert to the only food source left - the weakened or broken hydrocarbon substrate. The transition state complex is when the enhanced naturally occurring hydrocarbon degrading bacteria start converting hydocarbons to C02 and water.

The enhanced naturally occurring hydrocarbon degrading bacteria convert the solubalized hydrocarbons to C02 and water which is the end point or the Bioremediation of the hydrocarbon substrate. Any OSE II product components left are 100% biodegradable and will be used up naturally.

Dr. Venosa explains that having surfactants and emulsifiers preclude a product from being true Bioremediation. This is somewhat a misrepresentation of the facts, because in Mother Nature - when bacteria become proximal to a spill they release surfactants and enzymes to help breakdown hydrocarbon structures (detoxify) so the bacteria can utilize the spilled contaminant as a food source. OSE II has the same nutrients that Mr. Venosa pushes, plus we have all the constituents that occur in Mother Nature to speed up Bioremediation. To call Dr. Venosa's limited, and incomplete nutrients true Bioremediation over complete products that supply all of the constituents up front that are required by Mother Nature renders this fact sheet as nonfactual itself.

Paragraph 17.

OSE II is ideally suited for all applications - fresh or salt water - open water - beaches and marshes.

Chapter 18. Conclusions.

Mechanical cleanups (the method of choice) allows 80% of the oil to sink into the water. OSE II, on the other hand, FLOATS the oil, and rapidly detoxifies the oil, thereby protecting the ECO System and by rapidly Biodegrading the oil..

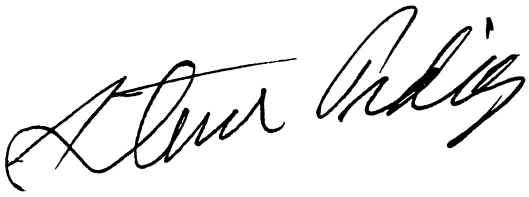
There are cost comparisons available and Dr. Venosa has this data. The Navy at the San Diego Fuel Farm has reduced their mechanical cleanup cost for oil spills on water from $90.00/spilled gallon to $12.00/spilled gallon and only $1.00 of the $12.00 cost is for OSE II.

CONCLUSION - BY: OSEI, CORP.

OSEI, Corp.'s OIL SPILL EATER II, solves all the problems spelled out in this Fact Sheet associated with Dr. Venosa's attempt to use and evaluate only nutrients.

OIL SPILL EATER II is successfully and effectively used on oil spills on soil and U.S. Navigable Waters.

OIL SPILL EATER II (OSE II) should be pre-approved by all RRT's for use on oil spills.



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WHY OIL SPILL EATER INSTEAD OF FERTILIZERS

1. HOW MUCH PRODUCT DO YOU NEED?

FERTILIZERS are mainly nitrogen and phosphorus which need to be continually re-applied (could be costly).

OIL SPILL EATER applications have been engineered so that for most cases, one (1) application is all that is needed - plus Oil Spill Eater has nitrogen, phosphorus and readily available carbon (fertilizer's do not). You know your cost.

2. CARBON SOURCE.

FERTILIZERS try to utilize the carbon from the hydrocarbons to enhance bacteria; the carbon is not always accessable. Since carbon, nitrogen and phosphorus are needed to enhance bacteria and growth, if the carbon is bound up, the fertilizer will take an extended period of time to react.

OIL SPILL EATER has an available natural carbon supply plus vitamins to readily enhance bacterial growth. By not relying on the carbon from the hydrocarbons, OSE will react better in a wider range of hydrocarbon contamination problems.

3. BACTERIA.

FERTILIZERS need for bacteria to already exist in hydrocarbon contamination areas in order to enhance biodegradation; and if the soil is innert, fertilizers may not work at all.

OIL SPILL EATER'S application, engineering and mixture ratios allow OSE to work whether soil is innert or not since OSE uses indigenous bacteria from the water used in it's application.

4. PRODUCT ADHERES TO OIL.

FERTILIZERS may be washed away from pollution site, which would render it useless. This washing away may also violate EPA'S new storm drain laws covering fertilizer, since high concentrations of fertilizers can also cause eutrophication.

OIL SPILL EATER has ingredients in the product to cause molecular adhesion of the product to the hydrocarbons eliminating the washing away of Oil Spill Eater.

5. CATALYST.

FERTILIZERS do not contain catalysts.

OIL SPILL EATER contains all the required nutrients, vitamins and catalysts in the form of enzymes. Enzymes act as catalyst to promote the enhanced bacteria to rapidly convert to feeding on the hydrocarbons.

6. SURFACTANTS.

FERTILIZERS depend on enhanced bacteria to produce enough surfactants to breakdown the hydrocarbon walls, so the bacteria can engulf the hydrocarbon itself. This is a slow process and requires enormous amounts of bacteria. For this reason fertilizers take a long time to show any reaction at all - assuming there are bacteria to start with.

OIL SPILL EATER contains various surfactants to help break the outer walls of Betx, light ends, aliphatics and even some asphaltines, and this allows the bacteria (a hydrocarbon ready) to be engulfed by enhanced bacteria, which reduces biodegradation time.

SUMMARY:

OIL SPILL EATER gives you the following benefits over fertilizers:

1. COST CONTROL - We know how much "Oil Spill Eater" II is required on each spill.
2. OWN CARBON SOURCE - OSE contains it's own carbon which aid in bacterial growth.
3. BACTERIA - OSE uses indigenous bacteria.
4. PRODUCT ADHERES TO OIL - OSE molecularly adheres to hydrocarbons.
5. CATALYST - OSE enzymes are catalyst for breaking down hydrocarbon walls and rapid bacterial growth.
6. SURFACTANTS - OSE contains it's own surfactants to help breakdown hydrocarbon walls.

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WHY USE "OIL SPILL EATER" II

RATHER THAN NON-INDIGENOUS BACTERIA

1. YOU CANNOT DIRECTLY APPLY ANY LIVING ORGANISM (BACTERIA) TO A TOXIC SUBSTANCE WITHOUT KILLING THE ORGANISM.

2. THE ONLY WAY FOR ANY BACTERIA TO UTILIZE THE HYDROCARBON OR CONSTITUENTS AS A FOOD IS TO FIRST REDUCE THE TOXICITY.

3. BACTERIA CAN UTILIZE MOLECULARLY REDUCED HYDROCARBONS AS A FOOD SOURCE, BUT ONLY AFTER THE MOST TOXIC COMPONENTS OF THE HYDROCARBON ARE REDUCED.

W H Y?

1. WHEN BACTERIA BECOME PROXIMAL TO A FRESH HYDROCARBON SPILL, THE FIRST THING THAT HAPPENS IS THE BACTERIA PRODUCE BIO-SURFACTANTS TO ALTER THE MOLECULAR STRUCTURE OF THE HYDRO-CARBON AND REDUCE IT'S TOXICITY. IF THERE IS TOO HIGH A CONCENTRATION OF BETX, THE BACTERIA DIE.

   IF THE CONCENTRATION IS DILUTED ENOUGH AND THERE ARE ENOUGH BACTERIA PRESENT TO PRODUCE ENOUGH BIO-SURFACTANTS TO KEEP FROM BEING OVERWHELMED BY THE HYDROCARBON, THEN THEY HAVE A CHANCE TO SURVIVE. USUALLY, THIS IS ONLY POSSIBLE IN A SITUATION WHERE A LARGE QUANTITY OF WATER IS PRESENT WHERE BACTERIA CAN GET CLOSE ENOUGH TO THE HYDROCARBON TO ATTACK AND YET SWIM AWAY IF NEEDED.

   IF THE BACTERIA ARE FORCED TOO CLOSE TO A TOXIC HYDROCARBON, IT DIES. IT TAKES A LONG PERIOD OF TIME FOR BACTERIA TO ACCLAMATE THEMSELVES TO A SPILL AND THEN ATTACK IT.

2. YOU CLEAN UP TOXIC SPILLS (HYDROCARBONS) BECAUSE THESE SPILLS CAN PREVENT LIVING ORGANISMS FROM LIVING. THEREFORE, SPILLS ARE CLEANED UP TO REDUCE OR ELIMINATE THE TOXICITY TO OUR ENVIRONMENT.

WHY USE "OIL SPILL EATER" II
RATHER THAN NON-INDIGENOUS BACTERIA

PAGE TWO

NON-INDIGENOUS BACTERIA LIMITATIONS:

1. DIRECTLY APPLIED TO A TOXIC SPILL THEY DIE.

2. IN THE ENVIRONMENT, INDIGENOUS BACTERIA GENERALLY TAKE OVER NON-INDIGENOUS BACTERIA.

3. NON-INDIGENOUS BACTERIA HAVE A HARD TIME ACCLAMATING TO A NEW ENVIRONMENT.

4. IF YOU HAVE 10°F OR MORE TEMPERATURE VARIATION, THE NON-INDIGENOUS BACTERIA GO INTO SHOCK AND THEN HAVE TO RE-ACCLAMATE THEMSELVES.

5. IF THERE IS A LIMITED AMOUNT OF NUTRIENTS IN THE SOIL, NON-INDIGENOUS BACTERIA WILL BE UNABLE TO SUSTAIN LIFE LONG ENOUGH TO ATTACK A SPILL.

6. YOU DO NOT KNOW YOUR COST! BACTERIA HAS TO CONTINUALLY BE ADDED UNTIL SOMETHING HAPPENS.

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USE OIL SPILL EATER II (OSE II) or ORC

USING ORC ONLY

ORC is an oxygen- releasing compound. ORC can only be effective in an area where there has been a hazardous material spill and (1) oxygen is the only limiting factor and (2) there is no means of injecting oxygen through a hose from a compressor or some type of commercial blower.

If Bioremediation precursors, nutrients, or bacteria are not present, then adding ORC will have limited benefits. Without Bioremediation precursers (which are present in OIL SPILL EATER II) (OSE II), then Bioremediation will be extremely slow even with the addition of ORC.

In any system where BOD or COD or utrified water might be a problem, ORC might be a benefit. However, it seems simple to set up a gasoline compressor or electric blower and blow air through a hose into the oxygen deficient zone.

USING OIL SPILL EATER II

OIL SPILL EATER II (OSE II) Concentrate is a liquid nutrient with enzymes that is mixed with water and applied to hydrocarbon spills or organic contaminants. OSE II grows indigenous bacteria using its nutrient material while enzymes form binding sites on the hydrocarbon walls. After eating OSE II Nutrients, the Bacteria then attack the hydrocarbon or contaminant; turn it into Carbon dioxide and water.

The limiting factors in remediating spills are the precursors (Bio-surfactants and Enzymes) in the correct mix of nutrients and constituents to carry out cellular metabolism, as well as cellular reproduction.

If these constituents are not present, then oxygen addition (ORC) will have little affect on a spilled material. An oxygen deficient situation is easily addressed; however knowing how much of the correct and complete constituents needed for Bioremediation is a much more difficult task to accomplish.

OSE II makes remediation a simple solution to a complex situation.

SUMMARY

Once again, the only time ORC can be a benefit is where oxygen is the limiting factor and there are nutrients available and air cannot be injected with some type of blower system.

The more efficient process is to apply OIL SPILL EATER II.

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Why OSE II Instead of
Surface Washing Agents (Soaps)

Surfactants wash the hydrocarbons (oil) off soil, concrete, rocks or asphalt, and simply moves your problem to a different area.

Surfactants (soap) merely break the hydrocarbons (oil) into smaller droplets which allows the hydrocarbons (oil) to sink or move and can be washed to a different area.

Surfactants, after washing or sinking hydrocarbons to a different area, can allow the hydrocarbons (oil) to recombine or reform in the new area.

Surfactants do not eliminate, remediate, or permanently solve the problem.

Oil Spill Eater II bioremediates the hydrocarbons to CO_2 and water, eliminating the problem in place.

Surfactants do not contain nutrients, enzymes, vitamins or constituents to complete metabolic life cycles, so it is impossible for surfactants to solve the problem in place.

Oil Spill Eater II has all the constituents to cause complete and rapid bioremediation of hydrocarbons.

Why spend money moving a problem when you can use **OSE II** and solve the problem where it is!

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WHY OSE II RATHER THAN ABSORBENTS

I. There are absorbents designed for water and absorbents designed for solid surfaces.

II. Absorbents designed for water are predominantly pads and absorbent boom.

1. It is almost impossible to put enough absorbent boom or pads out to collect large spills.

2. For even small spills pads are difficult to put in place to absorb moving spills, and then to pick it up before it sinks.

3. Absorbent pads or absorbent booms, once saturated, have to be collected and stored temporarily on shore, and then hauled away. Then you have to pay to dispose of them while exposing workers to hazardous material who directly lay the pads out and collect them.

4. It is also very difficult for absorbents to absorb viscous oils.

5. Absorbents require a considerable amount of labor for a minimum cleanup.

6. Once again, absorbents move the problem; they do not solve it.

III. Absorbents for solid surfaces can range from kitty litter to peat moss to pads.

1. To pick up spills on concrete or asphalt is difficult because absorbents have a hard time pulling a spilled material out of the pours of the surface.

2. Anytime it rains after absorbents have been used on concrete, you can see a sheen floating up.

3. It requires a lot of hands-on labor to put the absorbent out, wait for the absorbent to absorb (which in some cases puts workers in proximity of a fire hazard), then pick the absorbent material up, haul it away, then store it, then pay to have it disposed of.

4. This is moving the problem, after performing an incomplete cleanup.

IV. Recycling Absorbents

1. Some absorbents can absorb when you collect the pad or boom then take it to an area to wash or squeeze the absorbent, then store the absorbent. They may still contain some of the hazardous material (potential fire hazard).

2. This requires a lot of direct exposure by the worker/laborer to hazardous materials for an incomplete cleanup system.

3. This is a labor intensive system that, once again, moves the problem, it does not completely solve the problem.

V. Overview of the simple process using OSE II

1. OSE II has bio surfactants, enzymes, and a complete nutrient system to carry out complete metabolic processes (that emulate mother nature) to rapidly convert hazardous spilled material to CO_2 and water.

2. Water Spills

   A. When OSE II is applied to a water spill, the bio surfactant rapidly emulsifies and solubilizes the spill (detoxifies the spill, reduces the fire hazard, and breaks down the spill's ability to adhere to anything).

   B. There are constituents in OSE II that cause the spilled material to float so it does not increase the area impacted by the spill.

   C. Enzymes form digestion binding sites to be utilized by the rapidly grown indigenous bacteria who then use the spill as a food source and convert it to CO_2 and water. This is the exact process mother nature uses to address spills. OSE II has the required precursors to speed up the bioremediation process.

3. Concrete or Asphalt Spills

A. When OSE II is applied to concrete, asphalt, even soil, the bio surfactants and enzymes actually lift the entire spill leaving no residue to form a sheen when it rains. This also removes the potential fire hazard from the spill or from residue that could be left behind by absorbents.

B. In the case of airports where getting refueled planes out of a terminal and in the air quickly is important. If there was a fuel spill, simply spraying on OSE II, waiting approximately 3 minutes, then you can okay the plane to depart. With labor intensive absorbents, they would increase a plane's time at the terminal while not completely removing the fire hazard.

C. Once OSE II is applied, the process in #2 above would start and in 20 to 30 minutes you could simply wash the detoxified harmless spill residue away.

D. OSE II has constituents to cause it to molecularly adhere to spills, so wherever a spill is washed away to or current or wind carry it to, OSE II would stay attached and continue the bioremediation process until the spilled material is converted to CO_2 and water.

E. OSE II solves the spill problem effectively and in place.

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WHY USE OSE II INSTEAD OF COREXIT OR DISPERSANTS

I. Corexit is a dispersant.

II. Dispersants generally break oil into smaller droplets and then sink the oil / hazardous material it is applied to.

1. By breaking the oil into smaller droplets, this also spreads the spill contaminating a larger area than the initial spill itself.

2. When using Corexit, this also means you are spreading Corexit with the oil.

3. The oil / hazardous material then is caused to sink the oil into the water column.

    A. To be listed on the U.S. EPA National Contingency Plan for oil spills as a dispersant, it requires that the dispersant has to sink 45% of the oil in 30 minutes.

4. This hazardous material that is now spread out then sinks into the water column contaminating the oceans lower depths. This is creating a secondary area of contamination affecting fish, mammals or any species that survives or feeds in this area of the water under the surface.

5. The oil then settles on the oceans' floor. Now it contaminates the ocean floor with a hazardous material affecting bottom dwelling species and potentially killing them.

6. The sunken hazardous material then is swept along the ocean floor by underwater currents, contaminating expanding areas of the ocean floor and adversely affecting more numbers of living organisms.

7. This movement of sunken oil then starts to roll over and this oil starts attaching itself (recombining to some extent) to each other forming tar balls. These tar balls then roll up on beaches now affecting species of

organisms that live and feed in intertidal zones. In the case of sandy beaches you then expose humans to these somewhat toxic sticky tar balls. The oil in a tar ball state would then persist for a protracted amount of time.

8. Affected species with dispersants use:

   A. Species that live on the surface or feed on the surface of the ocean.

   B. Species that live or swim in the water column.

   C. Species that live on the bottom or forage for food on the ocean floor or spend some time on the ocean floor.

   D. Species that live in intertidal zones or travel through intertidal zones.

   E. In the case of sandy beaches, humans become exposed to this original surface spill.

   F. Any human being that comes in contact with dispersants that contain toxic solvents.

III. Toxicity

1. Most dispersants are a makeup of surfactants (a type of soap) and solvents. This makes the dispersant very toxic to living organisms.

2. In the case of Corexit 9527, the solvent utilized is ethylene glycol monobutylether (2 Butoxy - ethanol).

   A. Ethylene glycol monobutylether is so toxic that overexposure to your skin may cause kidney failure and eventually death.

   B. It is unfathomable that anyone would allow this or purposely apply this to the environment!

   C. In the EPA's NCP Product Schedule, the toxicity data for Corexit only is:

      (1) 9527 - Inland Silversides - LC50 14.6
      (2) 9500 - Inland Silversides - LC50 25.2

      Toxicity for Corexit's No. 2 Fuel are:

      (1) 9527 - Inland Silversides - LC50 4.49
      (2) 9500 - Inland Silversides - LC50 2.61

D. During an oil spill in the Gulf of Mexico, Corexit was accidentally sprayed on a Coast Guard ship. As reported to us by the U.S. EPA, the Corexit droplets dissolved the paint on the Coast Guard vessel.

We can only imagine what happened to any of the personnel that came in contact with this Corexit overspray.

E. Corexit's ethylene glycol monobutylether is a potential carcinogen. It is not listed as a carcinogen because it has never been tested for its carcinogenicity.

IV. Dispersant Summary

1. Using dispersants increases the areas impacted by the oil spill.

2. Using dispersants increases the toxicity of the oil spill.

3. Dispersants sink the problem into the water column, ocean floor, and beaches. They do not eliminate the problem; they simply move it.

V. Summary for Corexit Dispersants

The statements below are taken, (verbatim) from the *NALCO/Exxon Corexit Products Bulletin* from Corexit Dispersants "Material Safety Data Sheets" (MSDS).

These adverse effects to humans and the hazardous handling warnings described are NALCO/Exxon's own statements taken from the above publication.

A. Corexit 9500, 9527 and 9580

1. "*Caution: If unconscious, having trouble breathing, or in convulsions, do not induce vomiting.*"

B. OSHA statements about Corexit 9500, 9527 and 9580, are quote "*Based on our hazard evaluation, the following ingredients in this product are hazardous:*"

1. "Corexit 9500: Hydrotreated lite distillate - is a skin irritant, and TWA is 5 mg/m^3 ACG1H/TLV"

2. "Corexit 9527: 2-butoxyethanol is an irritant, systemic effects, combustible"

3. "Corexit 9580: Hydrotreated lite distillate - is a skin irritant, and TWA is 5 mg/m^3 ACG1H/TLV, STEL 10 mg/m^3 OSHA/PEL"

4. "Corexit 9500 - immediate (acute) health hazard"

   "Corexit 9527 - immediate health hazard, chronic health hazard, and a fire hazard"

   "Corexit 9580 - immediate health hazard and a fire hazard"

C. International Regulation:

"This is a WHMIS controlled product from the ingredients disclosure list or has been evaluated based on its toxicological properties, to contain the following hazardous ingredients:"

"9500 - hydrotreated light distillate"
"9527 - 2-butoxyethanol"
"9580 - hydrotreated light distillate"

D. Questions and Answers
(These statements are, again, taken from the *NALCO/Exxon Product Bulletin).*

"A committee of scientists for the National Research Council concluded in a 1989 report that the overall impact of spilled oil is <u>likely</u> to be reduced by dispersion!"

This statement *"that the overall impact is <u>likely</u> to be reduced by dispersion,"* is the only scientific data presented by Exxon. *<u>"Likely"</u>* does not mean it will *<u>"absolutely"</u>* lessen the impact.

We have been unable to find any scientifically valid tests to prove this. Common sense will tell you that when you add an extremely toxic non-aromatic hydrocarbon to a spill you are increasing or adding to the impact of the spill.

Where is the scientifically valid data supporting Exxon's following claim (See 2 below):

1. When describing these dispersants, Exxon very carefully did not mention the extreme toxicity of the non-aromatic solvents that are a significant part of the chemical makeup of the dispersant.

2. *"Once dispersed as fine droplets, the oil is <u>readily</u> biodegraded by micro organisms in the sea."*

OSEI Corporation Comment:
Common sense should convince anyone that adding the toxic non-aromatic hydrocarbons found in Corexit's products to an oil spill would actually prevent and delay any biodegradation of the oil.

(If Corexit is lethal to humans, single cell organisms have no chance to use it as a food source.)

3. *"Dispersing the oil into the upper three meters of the water column keeps the oil from impacting the shoreline."*

OSEI Corporation Comment:
This is a misleading statement suggesting that the oil somehow remains "levitating" in the upper 3 meters of the water columns!

Once the oil is dispersed and begins to sink, there is nothing to *"magically"* hold the oil in the upper three meters. These toxic oil droplets descend to the ocean floor adversely impacting everything in their path.

The end results could be tar balls that so readily cover Gulf Coast beaches.

Exxon claims that *"this process results in a net environmental benefit."*

OSEI Corporation Comment:
We do not agree with this claim since their toxic dispersant is spreading the now very toxic oil which can cause adverse impact on all areas of the ecosystem.

4. *"When properly used, Corexit products are of very low toxicity to marine life and humans."*

OSEI Corporation Comment:
See Section III - 2 - C on Toxicity. With toxicity values of 4.49 and 2.61 with No. 2 fuel oil, it is hard to comprehend their claim of low toxicity to marine life and humans!

VI. Why you should use OSE II

1. OSE II rapidly emulsifies and solubilizes the spill (detoxifies it) while reducing it as a fire hazard and lessens the spill's toxic impact immediately.

2. Once emulsification and solubilization are complete, the oil will not adhere to birds, mammals, any species, wood, metal, sand, soil, rocks, ships or humans.

3. OSE II has all the constituents to rapidly grow bacteria and carry out all metabolic processes so the oil is rapidly converted to harmless CO_2 and water.

4. OSE II causes the oil to float so only the surface is impacted by the spill (which the spill impacted anyway). Thus, there is no secondary impact to the water column, no impacting of the ocean floor, or impacting of intertidal zones.

5. An associate of OSEI Corporation drank 2 ounces of OSE II on a Houston, Texas television station to prove it is non-toxic.

6. Toxicity tests on Mysids when performed by the U.S. EPA proved OSE II to be virtually non-toxic.

7. OSE II solves (remediates) the spill in place. It does not move the problem to another area. (OSE II emulates mother nature exactly).

OSEI Corporation Comments:

We find it difficult to understand why anyone would use dispersants on oil spills when there is a safer, more effective product available.

We find it even more difficult to understand why anyone would use Corexit Dispersants due to their hazardous, toxic and life threatening ingredients as indicated in their own publication.

Based on the adverse characteristics and hazards associated with dispersants, using OSE II is the clear choice for hazardous spill cleanup.

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SUMMARY

"BACTERIAL BIODEGRADATION OF HIGH MOLECULAR WEIGHT POLYCYCLIC AROMATIC HYDROCARBONS" OR WHY OIL SPILL EATER II WORKS SO EFFECTIVELY

The University of North Carolina (Department of Environmental Sciences and Engineering), through a Grant from the U. S. Geological Survey (Grant 14-08-0001-G2103) and the UNC Water Resources Research Institute, scientifically proved that PAH's (Polycyclic Aromatic Hydrocarbons) can be Bioremediated.

These tests not only showed the Bioremediation of PAH's, but they verified The pathways and constituents needed to Bioremediate PAH's.

These pathways and constituents have been discussed in Oil Spill Eater II's Literature since 1989. This study verifies the fact that bacteria cannot be put In direct contact with a toxic contaminant without suffering a great amount Of mortality.

The study discusses that when bacteria become proximal to a contaminant, The bacteria release enzymes and surfactants to start the breakdown of the contaminant and to open pathways so the contaminant might be used as a Food source for the bacteria.

These are exactly the steps described in OSEI, Corp.'s literature.

The study also addresses the needs to have certain constituents present to act as a catalyst to induce bacteria growth and bacterial metabolism of PAH's. OSEI Corp.'s literature has addressed this since 1989.

This study proved the fact that bacteria will die if brought in direct contact with a contaminant. Bacteria need to release enzymes and surfactants to utilize contaminants as a food; and that various constituents are needed to promote the rapid colonization of bacteria that can utilize a particular contaminant as a food source.

OSEI, Corp.'s chemical process and general description has covered the methods and processes that must occur for the rapid growth of bacteria and the steps that much occur prior to a bacteria being able to utilize contaminants as a food Source.

This study backs up OSEI Corp.'s literature and proves that the very toxic PAH's can be Bioremediated as long as the correct constituents are available and the correct process occurs.

OIL SPILL EATER II contains these necessary ingredients and performs the correct process required to Bioremediate PAH's or any other organic based contaminants.

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Bacterial Biodegradation Of High Molecular Weight Polycyclic Aromatic Hydrocarbons

Michael D. Aitken, Shu-Hwa Chen, Chikoma Kazunga and Randall B. Marx

Introduction

Polycyclic aromatic hydrocarbons (PAH) are among the most common pollutants found at contaminated industrial sites in the U.S. and, in fact, around the world. Although PAH are naturally occurring compounds and essentially ubiquitous at low concentrations in the terrestrial environment, high concentrations of PAH are found in contaminated soils at wood treating facilities, at sites formerly used to produce manufactured gas, and at petroleum processing operations. There are a wide range of PAH, from the simple two-ring compound naphthalene to large multi-ring compounds. We arbitrarily define those PAH with two or three rings as low molecular weight (LMW) species, and those with four or more rings as high molecular weight (HMW) species. One of the characteristic features of PAH contamination is that there is always a complex mixture of PAH, often in conjunction with a variety of other hazardous chemicals.

The U.S. Environmental Protection Agency currently regulates 16 PAH compounds as priority pollutants in water, and generally considers these same compounds as "total PAH" (tPAH) in contaminated soils. The 16 regulated PAH comprise both low and high molecular weight species, and seven of them are designated as known human carcinogens. All of the carcinogenic PAH (cPAH) are high molecular weight compounds.

In addition to their toxicity, PAH as a class are extremely hydrophobic chemicals. Naphthalene is among the most water-soluble of the PAH, and its solubility in water is only about 30 mg/L. The situation becomes much worse with increasing molecular weight: chrysene (a four-ring PAH) and benzo[a]pyrene (a five-ring compound) are soluble in water in the low part per billion (mg/L) range. By comparison benzene, normally considered to be a water-immiscible chemical, has an aqueous solubility of about 2,000 mg/L. Despite the very low solubility of PAH in water, PAH-contaminated materials have been shown to be toxic in a variety of bioassays.

Microbiologists have known for a long time that microorganisms can act on most of the PAH of concern. For this reason, PAH-contaminated soils and sediments are generally considered to be candidates for bioremediation. In many of the studies in which PAH degradation in contaminated soil or sediment has been studied, however, the higher molecular weight compounds have not been removed completely. We generally do not

exposed to a mixture of PAH, all of which are, to varying extents, structural analogues. Over evolutionary time scales such conditions would be expected to select for microorganisms with versatile metabolic capabilities. We also knew that organisms able to degrade but not grow on the higher molecular weight compounds needed to grow on something, so the low molecular weight PAH seemed like the best choice with which to begin.

We have studied each of the 11 bacterial isolates for their ability to grow on or degrade 13 PAH, ranging from two- to five-ring compounds. Most of the bacteria have a broad range of PAH substrates they can metabolize (Aitken et al., 1996). Of most interest in terms of maximizing the biodegradation of these compounds, however, is mineralization. So far we have studied the ability of nine of the strains to mineralize pyrene, chrysene, benz[a]anthracene and benzo[a]pyrene (the latter three compounds are all cPAH). The results of this work are shown in Table 1, which also summarizes the source and identity (if known) of each isolate.

Table 1. Mineralization of HMW PAH by Bacterial Cultures[a]

Strain[b]	Identification[c]	Mineralization			
		PYR[d]	CRY	BaA	BaP
G2	*Agrobacterium tumefaciens*	-	+	+	+
G3	*Pseudomonas saccharophila*	-	+	+	+
C5	*Pseudomonas cepacia*	-	+	+	+
R1	unknown	-	+	+	+
M6	unknown	-	+	+	+
P15	*Pseudomonas saccharophila*	-	+	+	+
P16	*Pseudomonas stutzeri*	-	-	-	-
P21	*Bacillus cereus*	-	-	-	-
VT1	*Pseudomonas paucimobilis*	-	+	+	+

[a] Cultures were first grown on phenanthrene, centrifuged and washed, then incubated with the test compound for 24 hours. All strains mineralized phenanthrene, the positive control.

[b] Strains G2 and G3 were isolated from soil collected at a refinery; strain C5 from a site used to treat railroad ties; strain R1 from a manufactured-gas plant site; strain M6 from a former wood treating site; strains P15 and P16 from another former wood treating site; and strain P21 from motor oil-contaminated surface soil. Strain VT1 was obtained from J. Fuglt at the University of Alberta, Canada.

[c] Identification was by fatty acid methyl ester (FAME) analysis by MIDI, Inc., Newark, DE. Strains designated as unknown did not match any strains in the MIDI database at the genus level.

[d] Abbreviations: PYR, pyrene; CRY, chrysene; BaA, benz[a]anthracene; BaP, benzo[a]pyrene.

There are two trends worth noting in Table 1. First, none of the organisms was able to mineralize pyrene under the conditions used in this experiment. Second, every organism either mineralized all three of the remaining high molecular weight substrates or did not

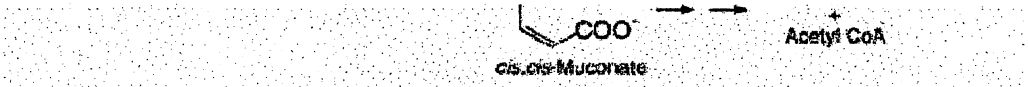


Figure 1. The principal pathway for aerobic metabolism of naphthalene by pseudomonads. Ring cleavage of catechol by either the *meta* or *ortho* route depends on the individual strain. Salicylate induces both the upper pathway (leading to salicylate) and the lower pathway (leading to catechol and subsequent ring cleavage reactions).

We have focused our attention on the inducibility of HMW PAH degradation in one of the organisms in our collection, Pseudomonas saccharophila P15. This organism was isolated several years ago by Will Stringfellow, who also conducted in-depth studies on its physiology (Stringfellow and Aitken, 1994; Stringfellow and Aitken, 1995). Both phenanthrene and salicylate induce the mineralization of benz[a]anthracene, chrysene, and benzo[a]pyrene by P. saccharophila P15 (Figure 2). None of the three compounds are used as growth substrates by the organism, so these results suggest that the metabolism of high molecular weight PAH in P. saccharophila P15 is linked to the metabolism of a low molecular weight compound. We do not yet know, however, if the different PAH are metabolized by a common pathway or if different pathways are regulated through a common mechanism.

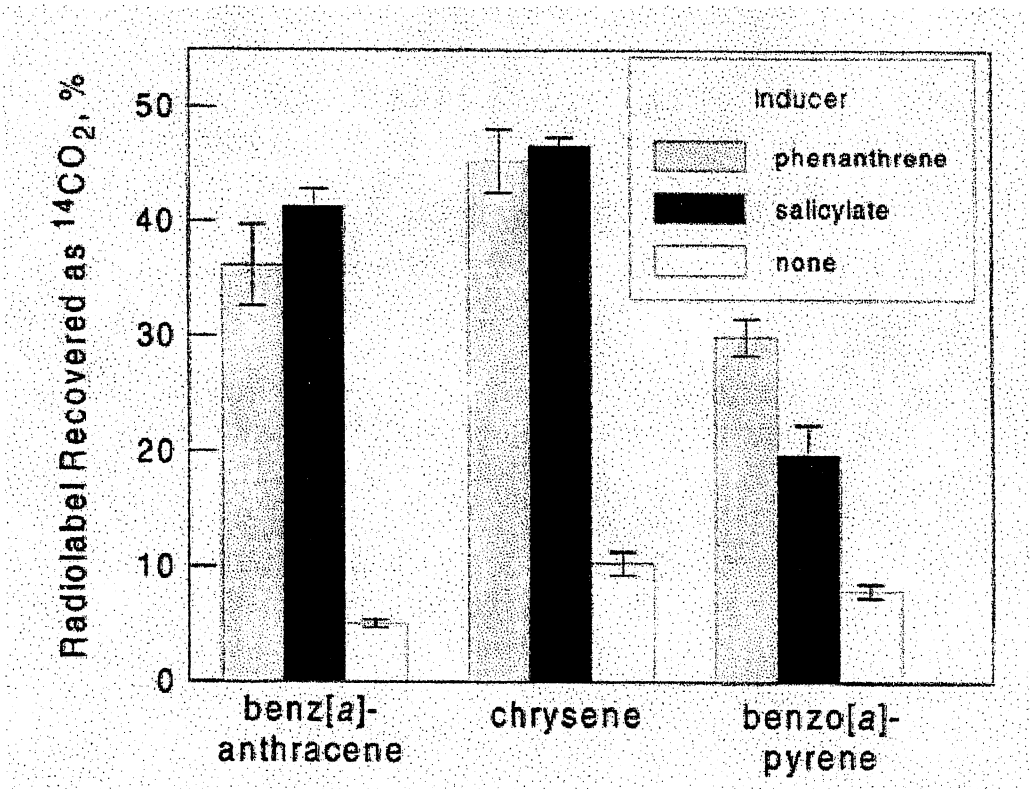
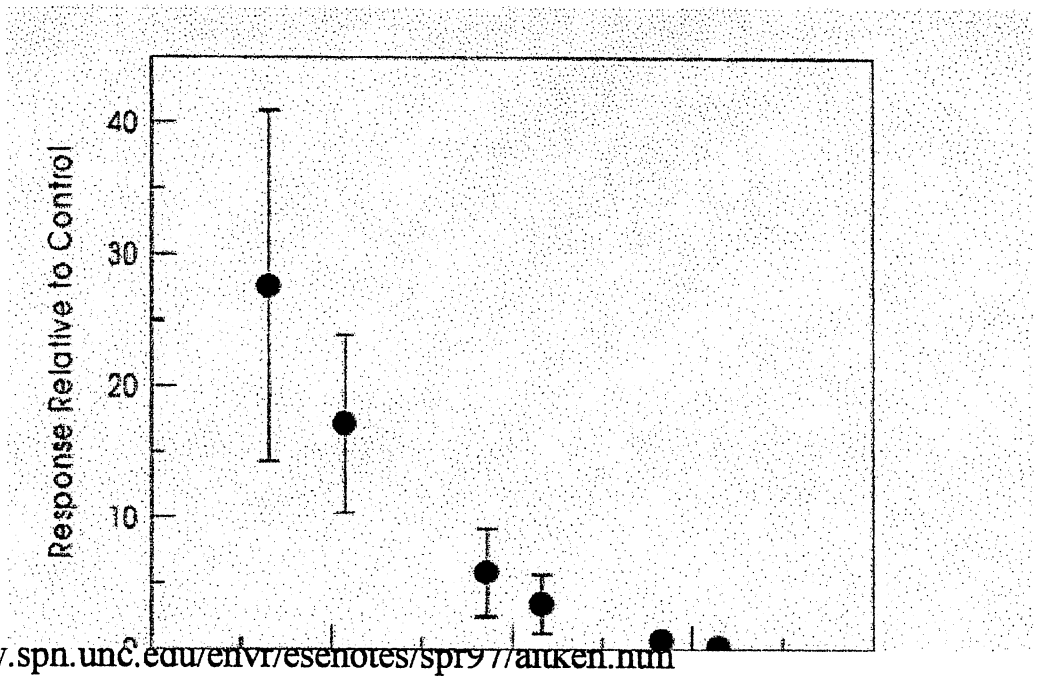


Figure 2. Mineralization of HMW PAH by *Pseudomonas saccharophila* P15. A culture of strain P15 was grown in a succinate medium, then incubated with the indicated inducer (or no inducer) at a 5 μM concentration for three hours. After the induction period, the cells were transferred to a medium containing radiolabeled benz[a]anthracene, chrysene or

such mechanisms in in situ biodegradation reactions and whether such mechanisms can eventually be manipulated to our advantage. If, for example, motility and/or chemotaxis are found to be important aspects of PAH (or other pollutant) biodegradation by soil bacteria, then we need to begin incorporating such phenomena into models that purport to simulate biodegradation processes in the subsurface.

Most of the bacteria identified in Table 1 are motile, and we screened most of them for chemotaxis to naphthalene, phenanthrene and known metabolites of these low molecular weight PAH. Only one of them, Pseudomonas stutzeri P16, seemed to have a chemotactic response to the chemicals of interest. Interestingly, strain P16 appears to be chemotactic to 1-hydroxy-2-naphthoate and to salicylate, both metabolites that have been found extracellularly in media containing active PAH degraders. However, we are learning that some of the methods traditionally used to study chemotaxis are not well suited to the study of chemotaxis towards compounds of low aqueous solubility. To test our methods with a known chemotactic organism, we recently obtained the strain Pseudomonas putida G7 from Professor Caroline Harwood at the University of Iowa, who recently identified this strain's chemotaxis towards naphthalene using a qualitative assay (reported at the 1996 American Society for Microbiology meeting). We learned that the chemotactic response of strain G7 in a standard quantitative assay becomes increasingly detectable as the starting concentration of bacterial cells is decreased (Figure 3). The optimum response we observed was at a cell concentration nearly two orders of magnitude lower than the concentration typically used in the assay, which we attribute to the rapid loss of the naphthalene concentration gradient at high cell concentrations and the necessarily low starting concentration of naphthalene. Overall the response of strain G7 to naphthalene was variable yet strong. Consequently, we will focus on this organism in our subsequent efforts to understand the relevance of chemotaxis to PAH biodegradation.



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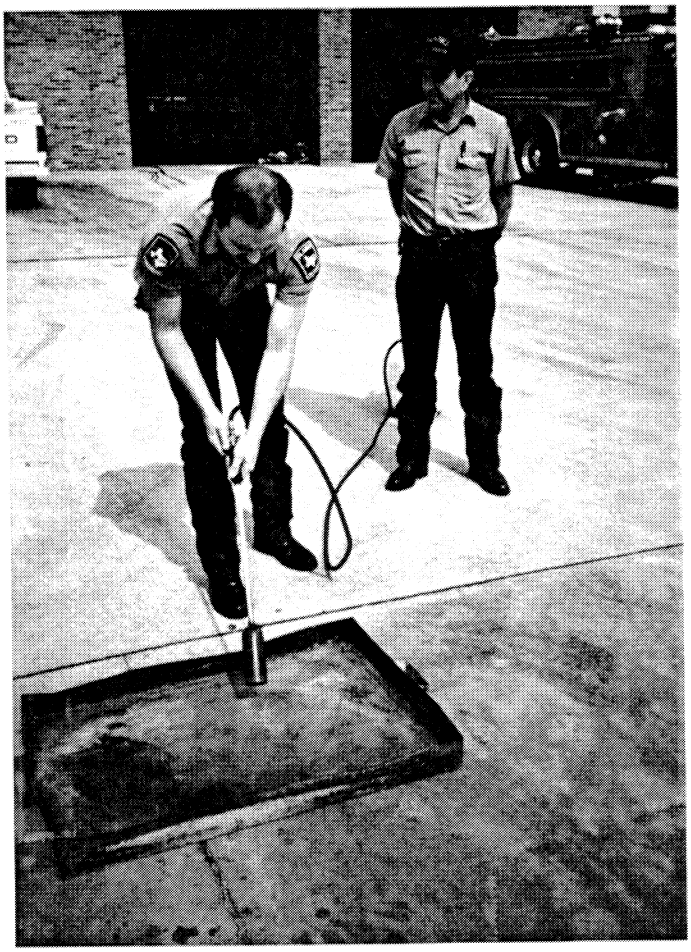
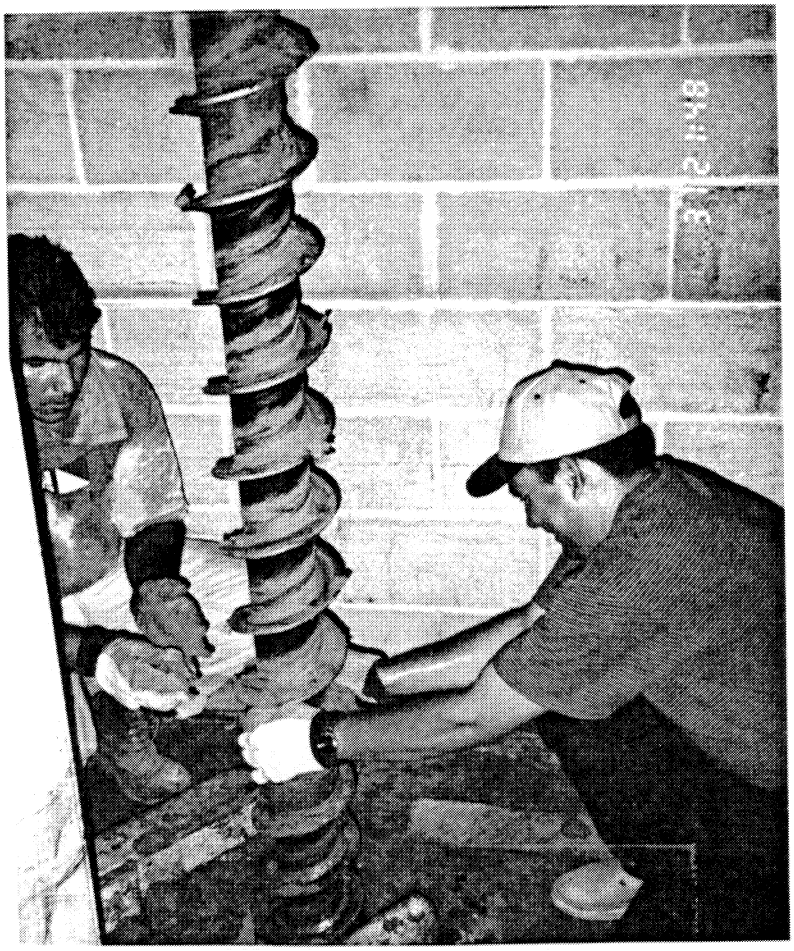
Department of Environmental Sciences and Engineering

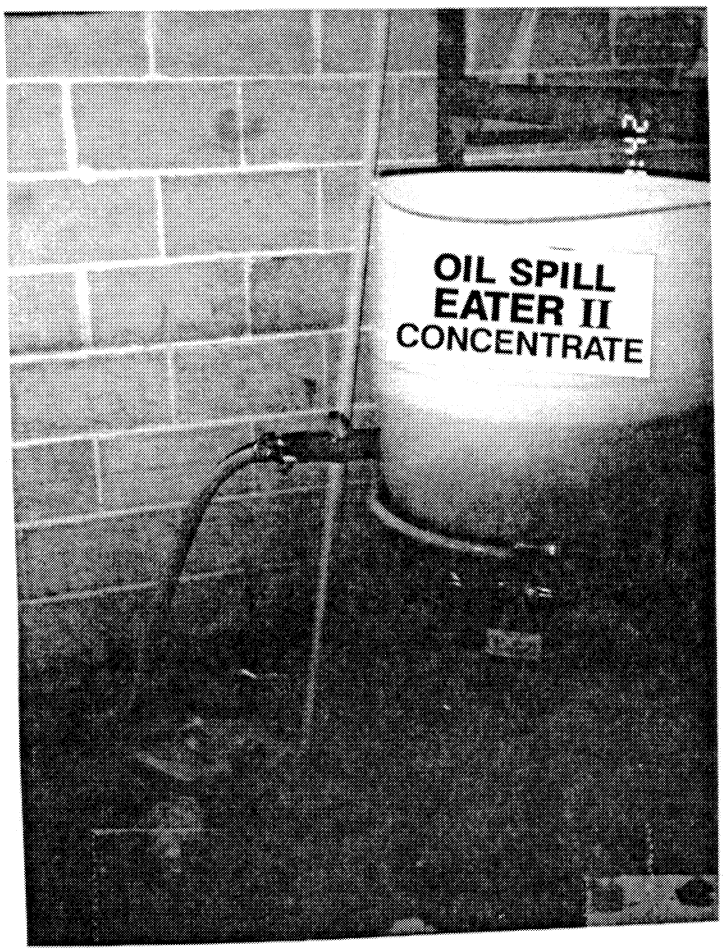
OSE II's TEXACO CRUDE OIL CLEANUP ON WATER



OSE II on DIESEL PROVING NONFLAMMABILITIES
3 MINUTES AFTER APPLYING OSE II

JET FUEL SPILL CLEANUP USING
OSE II AT JOINT RESERVE BASE - FT. WORTH, TEXAS





UNDER CHEMICAL PLANT CLEANUP USING OSE II -

Drilling Monitoring Well

Applying OSE II Down
Monitoring Well - 15' to 20'