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LAEO Environmental Solutions Institute Summary-Oil Spill Clean Up Technology

Bioremediation Enzyme Additive Category--Science Facts An Address of Common Questions

Enzyme-Based Bioremediation Oil Spill Removal Process

Bioremediation Enzyme Additive Category is a unique first response biological agent class, safe and effective for cleaning up every type of oil spill in all types of environments. It does not contain microbes of any kind. It breaks down the molecular structure of hydrocarbons through the effects of bio surfactants, followed by over 156 types of enzymes developing protein binding sites and acting as a catalyst, while enhancing indigenous bacteria to rapidly colonize. Once it's matrices and in particular, its nutrient portions, are depleted the enhanced bacteria convert over to the detoxified hydrocarbons as a food source via the enzymatic catalyst, digesting the hydrocarbons and hydrocarbon based material to CO₂ and water.

This technology has been around for decades and widely used. It emulates nature itself when she reacts to an oil spill, however greatly speeds up the process.

One developer of this biological agent type has a formula that is highly effective on all types of oil spills—and no other biological agent manufacturers have been able to duplicate the mix. It has been used for 25 years on more than 26,000 spills. This hydrocarbon removal cycle takes place in 14 to 30 days on most crude oils, and for very heavy long chained hydrocarbons, it can take up to 60 days from the time the remediation starts until full digestion of the hydrocarbons is complete. However, the toxicity and harmful aspects of a spill are eliminated within hours.

The Lawrence Anthony Earth Organization does not promote specific products or vendors. We do not financially benefit if we advocate for effective technologies—a necessity to ensure no conflict of interest. That said, we know of only a single source for the EA Category of Bioremediation—that is #B53 on the EPA NCP List-Oil Spill Eater II (OSE II).

Upon application of OSE II to an oil spill, several actions begin in a matter of minutes:

- a) the molecular structure of the hydrocarbons are broken down and therefore detoxified,
- b) the hydrocarbons are emulsified, then solubilized, which also causes hydrocarbons to float (when a spill is on water), or causes hydraulic lift producing a separation from other matter including plants, birds, etc.
- c) flammability of the hydrocarbons is significantly diminished, and
- d) adhesion properties are reduced to the point they will no longer adhere.

In other words, OSE II significantly reduces an oil spill's impact on the environment in a matter of minutes.

See video link <http://osei.us/archives/1135> for observations of what occurs when OSE II is applied to water or a sandy shoreline which can be instantly observed at the 9:40 time notation point on the video.

As the process continues, the bacteria deplete the OSE II matrices/nutrients and begin to convert over to the hydrocarbons as a food source, digesting the oil. The end point of the digestion process is merely a conversion of broken down detoxified hydrocarbons including all the attached OSE II matrices (which include its bio surfactants, enzymes and nutrients) to CO₂ and water. And, there are no intermediary constituents. This has been substantiated by independent literature reviewers and on real world spills, a recent 150,000 gallon spill in Nigeria at the Agip Brass Terminal which traveled 30 miles down sensitive delta channels – completely removed using OSE II.¹

The fact OSE II converts hydrocarbons to CO₂ and water has been substantiated by National Contingency Plan eligibility tests conducted by the US Environmental Protection Agency, on four separate occasions that we know of. The test reports showed that all 4 tests were within 10% of each others efficacy results, even despite the fact that one test was performed fifteen years after the first three previous tests. Further, these tests and many conducted by independent labs, government agencies and oil companies in other countries show all results consistent with US EPA's extremely thorough OSE II efficacy tests.

One such US EPA NCP test (see link <http://www.epa.gov/emergencies/content/ncp/products/oseater.htm>), was conducted with a control, and measures 54 analytes, or separate compounds of crude oil, to absolutely determine across the full spectrum of individual analytes if they are remediating, or mass is reducing. The tests are performed in triplicate, at 4 different times, day 0, 7, 14, and 28. These tests absolutely substantiate that OSE II is converts oil to CO₂ and water.

¹ Recent 150,000 gallon, or 550,000 liter clean up with OSE II in Nigeria see link <http://osei.us/wp-content/uploads/OSEI-Nigeria-AGIP-Brass-Terminal-Clean-up-complete-data-set-4-14-14-.pdf> also see video at link <http://osei.us/archives/1519>

LONG TERM STUDIES NOT REQUIRED

The notion that a long term study of “after effects” of OSE II is needed to determine long term environmental impacts is nullified and pointless once the conversion of hydrocarbons to CO₂ and water is complete. Because the hydrocarbons are removed from the environment, whether it be ocean, fresh water, estuarine, or soil environment; the biodegradable and organic ingredients of OSE II are no longer present after the process is complete. Monitoring protocols, however, have been developed and are prescribed for all types of environments as a matter of best practice.²

TOXICITY LEVELS = INSIGNIFICANT

In terms of concerns over toxicity to marine species, OSE II has had over 18 toxicity tests performed on it, some by the US EPA, Environment Canada, and in accordance with other country’s requirements. The average LC or LD 50 is 5000 and above. Keep in mind the US EPA has established anything above 100 to be virtually non toxic. See test at link <http://osei.us/wp-content/uploads/18-Toxicity-test-with-4-2012-Log0.pdf> page 25.

To address possible concern over intermediaries, the US EPA had Western University of Florida perform a toxicity test on OSE II, using it in a simulated open water test. The toxicity tests were performed on two different species over a 7-day time period. The 24 hour toxicity test on both species showed, an LC 50 of over 6000, the 48 hour LC 50 on the same species is 5970, the 96 hour LC 50 is 5700, and the 7 day LC50 is 2500. These toxicity tests tell that when OSE II is applied to oil, there is virtually no intermediate toxicity. In a second [and completely separate] species toxicity test, LC 50’s were 8839 for the 48 hour and the 96 hour test. These tests suggest there are no intermediate toxicity concerns even during the time frame hydrocarbons are being remediated to CO₂ and water.

A good example of long term and repeated use having no toxic effects is with an end user in Bakersfield California who has used OSE II to remove oil dust (from oil fields nearby) on a monthly basis from a 2600 gallon Koi fish pond, with abundant vegetation surrounding the edges of the pond. See video link at <http://osei.us/archives/1150>. This clearly demonstrates the application of OSE II to the oil dust on the surface of the water. The fish come up and appear to ingest some of the OSE II and swim through it several times, without harm. This application of OSE II has been done routinely for over two and a half years, without any harm to the Koi fish, in fact the Koi are thriving and growing as well as the plants and vegetation surrounding the pond.

² The conventional call for *long term studies* are derived from the use of chemical dispersants, since they have proven to disperse and sink oil into the water column with still unknown effects; although we do know that portions of the oil form into plumes or tar balls when dispersants are used, which also end up on shorelines and/or remain on the seabed.

Dispersants have proven to contain constituents that prevent or slow the degradation of hydrocarbons by indigenous bacteria. Dispersants have demonstrated a tendency to exacerbate the toxicity of the oil, to cause oil to persist in the environment, as well as to facilitate ingestion by marine organisms and life, again, with as yet unknown effects; hence, long term studies are required.

The U.S. Occupational Safety and Health Administration (OSHA) has reviewed OSE II and determined there is no toxicological concern for humans in regards to OSE II see link http://osei.us/tech-library-pdfs/2011/9-OSEI%20Manual_OSHA.pdf

OSEI Corporation demonstration videos generally show it to be harmless as persons can be seen pouring OSE II over their hands to clean any oil off. These types of demonstrations have been carried out repeatedly since 1989 thousands of times without any harm coming to the OSEI associates being exposed to the product.

We are not asking any RRT to believe what LAEO Environmental Solutions Institute says or publishes at this stage—we have been submitting documentation on this since the BP Gulf of Mexico oil spill which has not been thoroughly examined. What we are asking for at this stage is that the US EPA, NOAA and all other members of the National and Regional Oil Spill Response Science community LOOK at the data and field demonstrations.

SUMMARY

In summary, OSE II has an absolute pass score on its repeated efficacy tests with over 27,000 successful cleanups on its record since 1989 on fresh, brackish and salt water spills, as well as spills in sensitive habits, on soil, underground, on ground water and concrete, permanently removing the spills by converting them to CO₂ and water.

Being that OSE II is classified as an *Enzyme Additive Category Bioremediation Agent* on the US NCP Product Schedule (meaning it contains no microbes) and its clean up record, qualifies it as a first response tool on fresh and ocean navigable waters while other categories are not recommended as such. To understand why it is different than other types, see Bioremediation Fact Sheet revisions attached to this summary.

Attachment 1

Efficacy Documentation Summary **Regarding Oil Spill Response Agent *Oil Spill Eater II*--Listed on** **EPA NCP Product Schedule as number B53 *Bioremediation*** ***Agent Enzyme Additive [EA] Category***

5 May 2012
Updated 12 February 2014

The following documentation summary is based on LAEO and other expert's examination of EPA/NETAC & other credible efficacy and toxicity test reports, trials and long-term records showing successful field applications of Oil Spill Eater II (OSE II).

Since 1989, per reports from OSEI Corporation as well as independent testimony, *Oil Spill Eater II*™ (OSE II) an Enzyme Additive Category Bioremediation oil spill response agent (a sole sourced product as of the date of this writing), has successfully cleaned up more than 27,000 hydrocarbon based spills.

OSE II is distributed in more than 40 Countries, is listed on the US EPA's National Contingency Plan for Oil Spills (NCP Product Schedule) as well as listed in the U.S. Defense Logistics supply chain and the Navy DENIX system as BAA Book 18 number 14. (US Military have placed thousands of OSE II orders since 1992 <http://osei.us/defenseletter.pdf>) It is also listed and authorized for use in 17 other countries.

OSE II has undergone extensive efficacy and toxicity testing by EPA/NETAC and other credible institutions since 1989. In 2009, OSE II underwent a new set of efficacy testing based on EPA prescribed protocols with complete analysis done by LSU. A copy of the complete report is attached. (*Bio Aquatic Lab NCP Complete Testing*). This US EPA NCP test is summarized on the EPA website (see link <http://www.epa.gov/emergencies/content/ncp/products/oseater.htm>). This test was conducted with a control and an EPA prescribed Nutrient and OSE II. It measured across the full spectrum of 54 analytes to determine remediation and mass reductions. In summary, OSE II performed very well, reducing 89.9% of Alaska North Slope Crude within 28 days. These tests substantiate with certainty that OSE II effectively converts oil to CO2 and water.

See **Attachment 2**--literature review done by King Fahd University of Petroleum & Minerals, Research Institute, Center for Environment & Water.

Toxicity Testing Summary:

Western Florida University under contract from US EPA Hap Prichard in Gulf Breeze, Florida did simulated open water testing with OSE II on oil, measuring its efficacy and showed no acute or chronic toxicity; the 7-day toxicity test was above 2500. OSE II made it through three individual tier reviews by the 31 Scientist panel and moved onto Tier IV. See report at: <http://www.nbiap.vt.edu/brarg/brasym95/kavanaugh95.htm> and links below.

More than 20 credible [toxicity tests](#)ⁱ have been performed on OSE II validating this product as virtually non-toxic. The following chart using EPA and Environmental Canada numbers illustrates this pretty clearly:

Aquatic Toxicity (ppm*) Comparison--Bioremediation EA vs. Corexits						
Environment Canada Tests					U.S. EPA Tests	
Species	Oncorhynchus mykiss	Photobacterium phosphoreum	Gasterosteus aculeatus	Daphnia magna	Menidia (silverside fish)	Mysidopsis (shrimp)
Corexit 9500	354 (96hr)	0.065 (IC 50)	not listed	not listed	25.2 (96hr)	32.23 (48hr)
Corexit 9527	108 (96hr)	not listed	103 (96hr)	42 (48hr)	14.57 (96hr)	24.14 (48hr)
Bioremediation EA (OSE II)	10,000 (96hr)	5109 (IC 50)	not listed	10000 (48hr)	8839 (96hr)	6698 (48hr)
Higher # = less toxic, lower # = greater toxicity						
*expressed in terms of LC 50 values except for IC 50 where noted. LC 50=Lethal Concentration values in parts per million measuring level in which there is mortality with 50% of species being exposed over a specific period of time.						
Toxicity Comparison, Environmental Canada and U.S. EPA Tests, Bioremediation EA vs. Corexits (34)						

Other Countries:

OSE II has also been lab and field tested, certified and officially registered/listed by government regulators in 17 other countriesⁱⁱ including in the middle east under MEMAC within the ROPME Region and just recently by the Australian Maritime Safety Authority who conducted extensive efficacy and toxicity testing. See listing and test information at:

(<http://www.amsa.gov.au/environment/maritime-environmental-emergencies/national-plan/General-Information/control-agents/list/>)

OSE II can be safely and cost effectively used in any type of environment on oil spills and virtually any hydrocarbon based material including PCBs, wastewater treatment, groundwater contamination sites, harbors, airports, refineries and any oil or fuel spill prone location or storage

facility. It is also safe and non-toxic in sensitive habitats such as salt water marshes, fresh water environments, ocean and marine eco systems, rivers, inlets, public beaches etc.

LINKS TO OTHER EFFICACY & TOXICITY TESTS SUMMARIES

OSE II can be used on the surface, below the surface, on the ocean floor, in marshes, estuaries, and sand or soil, beaches on rocks, in bays, ports and harbors. Ample case studies in the field are available to prove it's workability in all mediums. OSE II is virtually non-toxic and extremely effective in breaking down oil and fuels. Samplings of OSE II toxicity tests are listed below and more can be found in OSEI Corps Technical Library OSEI Corporation's [Technical Library](#).

(to view documentation and actual test reports, click the blue links below or see attached.)

Salt Water Efficacy Tests:

- U.S. EPA / NETAC 21 Day & 28 Day Bioremediation Test - Biodegraded Alaskan Crude 98% in 21/28 days. (pg 25-35)
- U.S. Respirosity Test – EPA determined OSE II to reduce hydrocarbons by 98% and aromatics by 85% which was better than any other product tested. (pg 41-44)
- University of Alaska (Dr. Brown) PAH Test – Demonstrates that OSE II with mineral nutrients and hydrocarbons is **300%** more effective than without OSE II. (pg 45-49)
- Mega Borg Ship Spill in Gulf (South African Crude Oil) Test – In 216 hours OSE II lowered TPH from 100,070 ppm to 516 ppm for a 99.5% reduction. (pg 50-52)
- BETX Bioremediation Test- OSE II can even work well on Benzene, Ethyl Benzene, Toulene and Xylene ratios demonstrate the potential to biodegrade as much as 98%. (pg 53-56)

Fresh Water Efficacy Tests:

- Chevron Crude Oil Bioremediation Test- OSEII on Chevron Crude in 24 days reduced 95,200 ppm to 690 ppm or 99.8% effective on biodegrading this oil.

Soil Efficacy Tests:

- U.S. Marine Corps Base 29 Palms California (Cleanup Won Environmental Award) (pg 1-5)

Salt Water Species Marine Toxicity Tests

- U.S. EPA / NETAC Mysid Toxicity Test (this test was run twice) – LC50 Test, at 96 hours OSE II greater than 2100 mg/L.
- Both Mummichog and Artemia Salina Toxicity Test – LC50 Test, at 48 hours OSE II is 5285 mg/L. (pg 14-23)
- EPA/NETAC testing performed by University of Western Florida under contract from US EPA Hap Prichard performed toxicity testing with OSE II where in a simulated open water test OSE II was applied to oil and the effluent was tested on two different species and the average LC 60 was above 5000 showing OSE II is virtually non-toxic. The test also measured the degradation of the oil showing significant remediation.
- Toxicity testing from the above open water mesocosim effluent as well as toxicity testing the US EPA, Environmental Canada, South Korean and other foreign agencies have conducted prove OSE II is non-toxic to salt and fresh water species.

Fresh Water Species Marine Toxicity Tests:

- Rainbow Trout Toxicity Test by Environment Canada-Toxicity tests state 1000 mg/L or less is toxic. Anything higher is acceptable and considered non-toxic. OSE II, test result 10,000 mg/L = non-toxic.

Beneficial Environment Effects:

- Biological Oxygen Demand for OSE II –OSE II has minimal impact on BOD, less than 7%.
- Dispersant Swirling Flask Test - Proves OSE II causes oil to float

All this material is still applicable to the clarifications and changes in Clean Water Act NCP Sub-Part J protections that LAEO has been requesting for more than 3 years.

Copies of all this material can be downloaded in our NCP Revisions Information Center at: <http://protectmarinelifenow.org/take-action>

Thank You

ⁱ <http://osei.us/wp-content/uploads/18-Toxicity-test-with-4-2012-Log0.pdf>

Note, this document summarizes toxicity tests done on OSE II. The complete reports are available upon request and can be validated by contacting the 3rd party sources also. LAEO has taken the time to validate these as authentic and scientifically valid.

ⁱⁱ OSEI manufacturer has retained the right to keep the details of this information confidential to protect their business interests. Lawrence Anthony Earth Organization has examined the documentation from various countries where OSE II has been extensively used on major spills and can attest to the authenticity of the information but respect OSEI Corps request to keep this information confidential.