# REGION IV
# REGIONAL RESPONSE TEAM

# BIOREMEDIATION
# SPILL RESPONSE PLAN

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After a full scope of both laboratory and field testing, trials, demonstration, and a track record of successful cleanups of over 23,600 oil spills as of May 17th, 2013, the EPA's National Contingency Plan Bioremediation sub-category type called Enzyme Additive has proven to be the only category on the NCP list which meets the requirements set out by the original NRT/RRT IV Bioremediation Spill Response Plan. It is the only category on the NCP list that fully achieves the expectations and requirements of the Clean Water Act and OPA 90 as an oil spill cleanup methodology. It is, therefore, integrated into this proposed revision to the RRT IV's Oil Spill Response Plan, allowing the RRT IV, in a full-scale oil spill emergency, to immediately utilize a non-toxic, *first response,* thoroughly effective cleanup methodology that has no environmental or public health "tradeoffs," with full documentation cited to support each claim. This plan is easily adopted by any RRT as usable for all spill types and environments.

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# I. INTRODUCTION

Biodegradation is a natural process in which microorganisms chemically alter and breakdown organic molecules into other substances - such as fatty acids, carbon dioxide and water - in order to obtain energy and nutrients. The basis for this process is relatively simple: microorganisms require minerals and sources of carbon, as well as water and other elements, to survive and function. The process can involve one step or a series of steps that proceed through the formation of molecules with successively fewer carbons. Generally, the extent to which a particular organic molecule is biodegradable and the rate of degradation depend on the molecule's structural characteristics (chain length, amount of branching, number and arrangement of rings, stereochemistry) and the environmental conditions (temperature, available oxygen, substrate).

Bioremediation is a treatment technology that utilizes biodegradation to reduce the concentration and/or toxicity of chemical substances such as petroleum products and other hydrocarbons. Because microbes capable of degrading hydrocarbons are commonly found in nature, most untreated hydrocarbon spills eventually are removed from the environment by microbial degradation and other processes. However, the time frames of nature's process can be anywhere from 5 – 20 years and longer necessitating enhanced bioremediation, which seeks to accelerate natural biodegradation processes by applying specially chosen enzymes, nutrients and/or microbes to spilled substances. The basic purpose of taking additional measures to clean up an oil spill rather than waiting for nature do the job is to remove toxicity as swiftly as possible from the environment so that living organisms can survive, With advances in this field, leaving any toxic compound for nature to remove carries a risk of adverse effects on living organisms, marine and other life that can be avoided.

Although microbes have been used extensively and successfully for many years to treat wastes and wastewater in controlled facilities, their potential as a tool for responding to spills of oil and hazardous substances in uncontrolled environments has only more recently received significant interest. (For additional information on bioremediation, refer to Appendix G [SEE TYPES OF BIOREMEDIATION, page 6.)

With three types of Bioremediation identified/classified on the NCP Product Schedule, and each having very distinct differences in their mode of action and applicability in a given environment, the definitions and descriptions for these must first be understood to put this guidance document in proper context. (See Section III, (page 6) for this information)

This document presents a plan for considering and implementing bioremediation, through either natural attenuation or enzyme/nutrient/microbe enhancement, as a response tool for spills in US Environmental Protection Agency (EPA) Region 4. It was developed through the coordinated efforts of EPA's Subcommittee on National Bioremediation Spill Response and the members of the Region 4 Regional Response Team (RRT), using EPA's Interim Guidelines for Preparing Bioremediation Spill Response Plans.

## II. PURPOSE

This document has a four-fold purpose:

1. To define the three Bioremediation Agent Types listed on the NCP Product Schedule
2. To outline a process by which Federal On-Scene Coordinators (OSCs) in Region 4 may request authorization or pre-approval to use bioremediation in response to spills of oil or hazardous substances (the authorization procedures presented are consistent with the National Oil and Hazardous Substances Pollution Contingency Plan (NCP));
3. To outline the types of information necessary to determine if bioremediation is feasible, provide as much of this information in advance as possible, and outline a mechanism for capturing information on bioremediation use for future decision making; and,
4. To describe how to implement a bioremediation activity and determine if bioremediation is working.

The document is intended to guide decision makers in evaluating the appropriateness of bioremediation in the cleanup strategy for a spill and in undertaking a bioremediation activity. Ultimately, decisions regarding the use of bioremediation must be based on the OSC's best judgment given the particular circumstances of the spill incident.

The RRT's Response Technology Committee will examine, on an as-needed basis, the information in this plan, consider any new advances in and additional experience with bioremediation, and revise the plan as appropriate. Recommendations for revisions should be submitted to the Region 4 RRT for approval. Upon, approval by the RRT, revisions should be incorporated into the Region 4 RCP and other local plans, as appropriate.

## III. TYPES OF BIOREMEDIATION, CATEGORY DEFINITIONS

### MODE OF ACTION IN OPEN WATER, MARINE AND FRESH WATER ENVIRONMENTS

It is important to differentiate the three types of bioremediation processes since their efficacy requires precise application parameters that vary in different types of environments. The limitations and decision points on usage have been covered extensively in previously issued materials but require more simplification, hence this guidance has been provided to simplify the decision-making processes.

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Essential facts stated in the ***May 2000 NRT SCIENCE AND TECHNOLOGY COMMITTEE-Fact Sheet: Bioremediation in Oil Spill Response*** are:

"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….

"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." [i]

**NCP PRODUCT TYPES LISTED:**

The Bioremediation Agent Types listed on the NCP Product Schedule are deliberately designated and appear as follows:

"1. Microbiological Cultures (MC)
2. Nutrient Additives (NA)
3. Enzyme Additive (EA)"

The first type (MC) constitutes a bioremediation process that utilizes non-indigenous (foreign) bacteria. While useful in controlled environments, a prevailing concern with these types of products has been that the introduction of foreign species might cause future problems that may not become apparent for some time. The second type, (NA) is those agents that

contain nutrients or fertilizers to support the microorganisms present in the spill environment. Both are designated as not applicable for open water environments. See 2001 EPA Guidance *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands* that extensively covers the usage of these two product types which need not be repeated here.

On the other hand, the third type is appropriate as a first-response tool in open water environments. Bioremediation (EA) Type has evolved in recent years and has been the subject of considerable technological advances with wide applicability for oil spill response in fresh, brackish, marine and open water environments with temperature ranges as low as 28 degrees Fahrenheit. The mode of action of this type will be covered in detail here.

**IMPORTANT CONTEXT**

The reason for oil spill cleanup is to reduce or eliminate the toxic components, thus enabling the survival of fauna and flora including single cell organisms in each niche of the food chain. Although today's dispersants eliminate the visual and other damaging aspects of the spill on the surface, the spill's toxicity problem has remained in the environment and at times been worsened by the addition of further hydrocarbons in dispersants. The goal of the bioremediation process is to convert oil/hydrocarbon-based material to $CO_2$ and water, thereby permanently removing oil/hydrocarbons from the environment and returning the affected spill area to the pre-spill conditions.

Herewith, the three main types of bioremediation are further defined along with their modes of action to help OSC's, federal, state, and local officials as well as responsible parties to understand and make more informed decisions about bioremediation agents when selecting appropriate oil spill response tools.

## CATEGORY TYPE ENZYME ADDITIVE (EA)

As covered, while NRT and RRT guidance addresses the (MC) and (NA) bioremediation types extensively in the 2001 *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands,*[ii] it does not sufficiently detail the mode of action of *Bioremediation Type EA*.[iii] Below are data to remedy this.

## ENZYMATIC AGENT DEFINITION:

Bio-catalysts designed to enhance the emulsification and/or solubilization of oil to make it more available to microorganisms as a source of food or energy. These agents are generally liquid concentrates, which may be mixed with surfactants and nutrients that are manufactured through fermentation. This type of agent is intended to enhance biodegradation by indigenous microorganisms.

## (EA) TYPE MODE OF ACTION:

**Enzyme Additive mode of action is applicable in open/moving water (fresh, salt and brackish), marsh/estuaries, shoreline and soil environments.** When applied, the non-toxic converters and bio-surfactants in Bioremediation Agent (EA) Type eliminate the classic appearance of an oil spill by emulsifying and solubilizing the molecular hydrocarbon structure and eliminating the adhesion properties of crude oil. This usually takes place within the first 5 - 30 minutes (depending on temperature). The emulsified oil continues to float near the surface thereby eliminating a secondary impact to the water column and seabed.

With the toxicity and adhesion properties eliminated, wildlife that may come in contact with the broken down hydrocarbons will not become coated in oil, and oil adherence to marsh, shorelines, sands, and manmade structures is eliminated. The flammability is eliminated in a short time (depending on temperature) protecting ports, harbors and drilling rigs from the potential explosion hazards associated with fuel spills.

A further action of bioremediation category EA, (there are numerous enzymes contained in the product's matrices) is that the enzymes then attach themselves to the hydrocarbons with the biosurfactants, developing protein binding sites, that act as a catalyst to speed up the bioremediation process by inducing enhanced indigenous bacteria to utilize the detoxified oil/hydrocarbons as a food source. The EA category also contains properties that cause all the constituents to remain in contact with the spilled oil/hydrocarbons in moving waters.

Over the next few days or weeks (again, depending on temperature), non-toxic nutrients in the Enzyme Additive type rapidly colonize indigenous

bacteria to large numbers. The colonized bacteria consume the detoxified hydrocarbon emulsion, digesting the spill to $CO_2$ and water, thereby permanently removing the oil/hydrocarbons from the environment and resulting in final water clarification. Without category (EA) assistance, this natural process may take up to 20 years based on Ixtoc and the Valdez spill studies.

**SHORELINES/MARSHES:**

When a spill has already made landfall or contaminated a marsh, category EA can be applied to lift the spill off the marsh grass (or sandy beaches and shorelines), limiting the time the spill can adversely impact these areas. The use of category EA does not deplete the O2 from water since the spill is held on the surface utilizing predominantly atmospheric O2.

There are no tradeoffs or deleterious effects with the Category EA response method.

There is no limited window of opportunity for the application of category EA. It is effective as a first response tool and/or when applied days, months or years after a spill. It can be used in estuaries, in open (salt) water and, moving fresh water in rivers and soil. Category EA can even be applied to oil that is lying on the seabed floor as long as the product can be brought into contact with the oil which will eventually lift it to the surface returning the seabed to pre-spill conditions.
As of the date of this writing, there is only one product on the NCP list that falls under this official Bioremediation Sub-Category, Agent Type EA classification: (B53-EA-OIL SPILL EATER II). Therefore all mode of action descriptions above are related to this single EA product. Any newly added EA type listings would require review and validation as fitting into this category with the same above characteristics.

## CATEGORY TYPE MICROBIOLOGICAL CULTURE ADDITIVE (MC)

As covered in NRT Science and Technology Guidance; "… *Bioaugmentation* is a process *"in which oil degrading bacteria are added to supplement the existing microbial population.*"

## MICROBIAL AGENTS DEFINITION:

Concentrated cultures of oil-degrading microorganisms grown on a hydrocarbon-containing medium that have been air- or freeze-dried onto a carrier (e.g., bran, cornstarch, oatmeal). In some cases, the microorganisms may be grown-up in bioreactors at the spill site. All commercially available agents use naturally occurring microorganisms. Some agents may also contain nutrients to assure the activity of their microbial cultures. This type of agent is intended to provide a massive inoculum of oil degrading microbes to the affected area thereby increasing the oil-degrading population to a level where the spilled oil will be used as a primary source of food for energy. Microbial agents are designed to enhance the biodegradation of oil at any location and would be most useful in areas where the population of indigenous oil degraders is small.

## (MC) TYPE MODE OF ACTION:

Bioremediation Agent Type (MC) mode of action utilizes non-indigenous bacteria with the objective to digest oil/hydrocarbons to CO2 and water.[iv]

Bioaugmentation is considered a '**polishing up'** or '**finishing**' response product in that it has low efficiency when applied to fresh oil because the toxicity levels kill the added oil-degrading bacteria.

When non indigenous bacteria are placed on or near weathered oil these bacteria attempt to release enough quantities of biosurfactants to detoxify the spill so the oil-degrading bacteria will not be adversely impacted by the spill's toxicity, enabling them to use the hydrocarbons as a food source.

The oil-degrading bacteria (both indigenous and non indigenous) produce enzymes to develop protein binding sites that permit the bacteria to convert the molecular structure of the hydrocarbons for use as a food source. This process requires a protracted amount of time.

While bioaugmented bacteria acclimate to a spill site, the temperature of the water and or environment, the PH, and the available nutrients, these and other associated and variable environmental conditions may produce adversity that cannot be overcome. These factors along with the unknown time frames associated with their acclamation process are at least partially

responsible for the past uncertainty associated with bioremediation (MC) type as a viable cleanup methodology.

The application of non-indigenous bacteria generally must be performed where there is very little water movement. Water movement causes the products to dilute to ineffective levels that are unable to stave off the natural competition from indigenous bacteria, and, thus, will not be in sufficient population numbers to produce enough biosurfactants and enzymes to start the breakdown of the molecular structure of the hydrocarbons for a food source. (Lab environments do not emulate this competitive environment; hence, particularly in any area of moving waters, the final outcomes are often uncertain.)

Next to the toxicity of the spill, the most difficult aspect of utilizing non-indigenous bacteria in a foreign environment is the natural competition from the indigenous bacteria that are already acclimated to the spill area; thus, they generally win out.

Bioaugmented bacteria developed specifically for fresh water must be used in fresh water settings only. Products containing saltwater bacteria can only be utilized in saltwater. (MC) Type is best used on closed and/or controlled environments and is not effective in open water environments.

The use of non-indigenous bacteria in most countries is not permitted due to the uncertain effects of allowing non-indigenous species to be introduced into sensitive habitats and environments.

## CATEGORY TYPE NUTRIENT ADDITIVE (NA)

As covered in NRT Science and Technology Guidance; ". . . this next category (NA)--biostimulation is a process *"in which nutrients, or other growth limiting substances, are added to stimulate the growth of indigenous oil degraders*."

## NUTRIENT AGENTS DEFINITION:

Bioremediation Agents containing nitrogen and/or phosphorous as the primary means to enhance the rate of growth of indigenous oil-degrading microorganisms.

This type of agent is intended to increase the oil-degrading biomass already present in an affected area to a level where the oil will be used as a primary source of food or energy. Because the natural environment may not have sufficient nutrients to encourage bacterial metabolism and growth, extra nutrients may be required. The purpose of this type of agent, therefore, is to provide the nutrients necessary to maintain or increase microbial activity and the natural biodegradation rate of spilled oil.

## (NA) TYPE MODE OF ACTION:

The NA mode of action involves the general use of nutrients or fertilizers that contain various volumes of Nitrogen N and phosphorous P. The nutrients are placed in conjunction to a spill, where they are expected to enhance the growth and colonization of indigenous bacteria. These bacteria need time to secrete biosurfactants to attack the molecular structure of the spill by solubilizing the oil/hydrocarbons, then emulsifying the spill, increasing the oil-water interface to detoxify the hydrocarbons to the point the enhanced indigenous bacteria can utilize the spill as a food source.

It can be very difficult to apply nutrients or fertilizer in a spill area with toxic oil and still be able to enhance bacteria. Much of the indigenous bacteria are destroyed by the toxicity of the spill initially. Because of the toxicity of the oil, this situation usually precludes the nutrients or fertilizer being capable of enhancing what is left of the indigenous bacteria.

It is also challenging to supply nutrients or fertilizers in a concentration to enhance bacteria without increasing the nitrogen levels to the point that it becomes deadly toxic to aquatic life. An additional problem is getting the nutrients or fertilizers to stay with the oil especially on or in moving waters.

The process of enhancing indigenous bacteria with nutrients and fertilizer and waiting for them to secrete biosurfactants and enzymes in order to start the bioremediation process takes a protracted period of time making (NA) type inappropriate as a first response agent.

Bioremediation category (NA) can be effectively used where there is little tidal flush, and where the oil has weathered so its toxicity is reduced to the point that indigenous bacteria can survive. This requires NA to be used only as a “polishing up” agent, with limited scope.

## A BRIEF NOTE ON PHYTOREMEDIATION

Phytoremediation has been defined as the use of green plants and their associated microorganisms to degrade, contain, or render harmless environmental contaminants.

Phytoremediation of petroleum hydrocarbons generally involves three major mechanisms: (1) degradation, (2) containment and (3) the transfer of contaminants from soil to the atmosphere.

For further information on applicability consult page 87 of **http://www.epa.gov/osweroe1/docs/oil/edu/bioremed.pdf**

### SUMMARY

The three types of bioremediation and their mode of actions as described above have been detailed here to help responders understand how these agents will interact with a spill. The different types and their mode of actions are clearly independent of each other, even though their end point in principle is the same; the ability to reach that end point, and the amount of time it takes to do so, is clearly different.

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# IV. APPLICABLE REGULATIONS

Legislation at both the federal and state level may affect decisions to use bioremediation. Existing regulations and policies that govern the use of bioremediation agents in response to spills in Region 4 are summarized in Appendix A.

# V. ROLES AND RESPONSIBILITIES

This section discusses issues relevant to managing the response to a spill, with particular emphasis to managing bioremediation activities.

### On-Scene Coordinator (OSC)

As per 40 CFR Section 300.120, USCG and EPA provide pre-designated OSCs that have overall responsibility for oil spill responses in the coastal and inland zones respectively. When considering or actually using bioremediation as a response tool, the OSC shall be responsible for ensuring that the requirements

set forth in this plan are properly followed and implemented. This includes notification, planning, documentation and monitoring of all bioremediation activities. Thus, the OSC, in conjunction with his/her contractors or a responsible party, will be directly involved in the cleanup effort.

### Federal Agencies

US Environmental Protection Agency - EPA, with their extensive technical expertise in bioremediation, may lend themselves to the OSC as a technical advisor. This expertise includes information on the ability of various bioremediation treatment techniques to degrade oil, their relative toxicity to a habitat and the expected rate of degradation. Typically, EPA provides the Scientific Support Coordinator for inland zone spills. In addition, EPA maintains laboratory facilities that may be used to run bioremediation related studies and analyses. As internal EPA guidance documents have recently been found to have inaccurate and missing information regarding the different modes of operation and types of Bioremediation,, and, based on this inaccurate data, EPA officials have made inaccurate statements, it is highly recommended that EPA advice regarding Bioremediation be considered as valid only after attestation to a full review of the information above regarding the three distinct subcategories of Bioremediation.

US Coast Guard - The USCG supplies expertise in oil spill response technology and incident command. Response support, through manpower or equipment, can be provided by the Strike Teams and the National Strike Force Coordination Center. Additionally, the USCG can assist with cost tracking and funding support from the Oil Pollution Trust Fund. 1

National Oceanographic and Atmospheric Administration - NOAA/HAZMAT provides Scientific Support Coordinators (SSCs) and their support teams. The SSC provides scientific advice to support the Federal OSCs in operational decisions that will protect the environment effectively, mitigate collateral harm, and facilitate environmental recovery. The NOAA/HAZMAT Scientific Support Team has extensive expertise in all scientific aspects of spill response and mitigation and vast experience with oil spill response and several applications of bioremediation in both operational and experimental use. Their expertise in biology, geomorphology, chemistry, and physical and coastal processes and their support can assist in the appropriate selection of bioremediation as a response technique and in its proper application. NOAA/HAZMAT also provides the Department of Commerce RRT member. The DOC RRT member provides advice and access to NOAA and DOC resources and expertise and serves as the point of contact for DOC/NOAA trustee issues. As internal EPA guidance

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1 (With a working knowledge of Agent Type EA, the USCG has recommended action with this agent type numerous times and has used it at their own facilities.) see link http://www.osei.us/pdf%20files/Coast%20Guard%20BP%20spill%20approval%201.pdf

documents have recently been found to have inaccurate and missing information regarding the different modes of operation and types of Bioremediation, and, based on this inaccurate data, NOAA officials have made inaccurate statements. it is highly recommended that any advice regarding Bioremediation be given only after attestation to a full review of the corrected information above regarding the three distinct subcategories of Bioremediation. See link: http://www.osei.us/pdf%20files/NOAA%20Charlie%20Henry%20final%201%2025%202011%20.pdf

Department of Interior - DOI has direct jurisdiction for the protection of resources on its own lands, as well as trustee responsibilities for certain natural resources, regardless of location. They can provide information concerning the lands and resources related to geology, hydrology, minerals, fish and wildlife, cultural resources and recreation resources. The DOI natural resource trusteeship also includes migratory birds, anadromous fish and endangered or threatened species and their critical habitats. The DOI has performed at least one comparison test between chemical dispersants, mechanical cleanup, and Bioremediation Sub Category Enzyme Additive. Test outcomes demonstrated no removal of hydrocarbons from the environment (per the Clean Water Act) by the chemical dispersants, that mechanical cleanup achieved between 2-8% hydrocarbon removal (consistent with historical results), and Bioremediation Sub-category Enzyme Additive removed 67% of the oil. Therefore, they have first-hand, reliable knowledge of the workability of effective Bioremediation. See link http://osei.us/pdf%20files/OSEI-Summary-of-Department-of-Interior.pdf

### State, Local and Tribal Agencies

State, local and tribal agencies have a distinct role and perspective during a response that impacts their own resources. Typically, these agencies can provide valuable information on the latest regulations, guidelines, water resource conditions, environmentally sensitive areas and public concerns. Therefore, any response effort should be carefully coordinated with impacted State and local agencies. See attached COMPILATION OF DOCUMENTATION OF TEST AND USE RESULTS WITH THE RRT

### Responsible Parties (RP)

Since the RP has firsthand information concerning the spilled material, the RP may request OSC approval for the use of bioremediation or the application of a bioremediation enhancing agent. The RP can initiate a bioremediation activity after the request is approved by the OSC following concurrence from RRT 4 and consultation with the impacted natural resource trustees. The OSC's request, on behalf of the RP shall be accompanied by a completed Bioremediation Use Authorization Form. Maximum cooperation and participation should be expected from the RP throughout the entire response and bioremediation activity. ensuring

that the RP is fully aware of available Bioremediation agents, particularly as a first-response method, that will not introduce further toxicity to the already toxic spill site that, thereby, increase the RP's liability for damage done.

# VI. DECISION TOOLS

Spills may be good candidates for bioremediation treatment based on characteristics of the spill and environmental sensitivities of the spill location. To assist OSCs and the RRT in evaluating spills for bioremediation treatment and to document the basis for response decision making, the following are provided: (1) a diagram outlining the decision process that OSCs should follow when deciding whether to use bioremediation, and (2) a form for obtaining authorization to use bioremediation that specifies information which should be collected for presentation to the OSC and RRT. This form, the Bioremediation Use Authorization Form, is presented in Appendix B, page 49.

### Decision Process

Decisions to use bioremediation should be made after applicable regulatory policies, potential environmental impacts, operational feasibility, logistical coordination, and other pertinent issues have been evaluated. Ideally, these steps should have been performed prior to a spill occurring so that the RP and involved agencies can immediately move into effective action to remove the toxicity swiftly from the environment when a spill occurs. As Bioremediation Sub-Category Enzyme Additive is the only first response Bioremediation method, and one that can be safely and effectively applied in any environment, with proper pre-planning, logistical coordination is the only issue that should have to be dealt with at the time of an actual spill. The process to determine whether bioremediation may be feasible for a particular spill is illustrated in Diagram 1 (page 17). Details for addressing the specific issues are outlined in the section Feasibility Assessment Criteria (page 19).

### Bioremediation Use Authorization Form

A Bioremediation Use Authorization Form that specifies the minimum information requirements necessary to support decisions regarding the use of bioremediation is included in Appendix B (page 49) of this plan. The form requests details of the spill incident, bioremediation details, bioremediation Work Plan and Monitoring Plan. Once the form has been completed, it should provide pertinent information needed to make a decision regarding the use of bioremediation.

A completed authorization form should be transmitted to the RRT for the required authorization to proceed with bioremediation treatment. The RRT shall approve or disapprove the use of bioremediation within *24 hours* of receiving a completed form from an OSC.

**DIAGRAM 1: STEPS TO TAKE AND QUESTIONS TO CONSIDER DURING DECISION PROCESS FOR USE OF BIOREMEDIATION**

**DIAGRAM KEY**

**NA = Nutrient/Fertilizer Additive**
**MC = Microbial Agents**
**EA = Enzyme Additive**
**TBD – To Be Determined**
**LTD – Limited potential use**

| QUESTION | INFORMATION |
|---|---|
| **IDENTIFY SPILLED POLLUTANT** | |
| **A) HYDROCARBON-BASED?** | Yes _____ No _____ |
| **B) FRESH SPILL?** | Yes _____ No _____ |
| **C) WEATHERED?** | Yes _____ No _____ |
| **1. WILL BIOREMEDIATION EFECTIVELY ADDRESS THIS POLLUTANT?** | **NA Type:** TBD: If fresh, no. If weathered, LTD; risk of excess nitrogen levels killing aquatic life.<br>**MC Type**: TBD: If fresh, no. If weathered, LTD; risk of introducing non-indigenous (foreign) microbes creating future unknown problems.<br>**EA Type**: Effective on both fresh and weathered. |
| **2. DO REGULATIONS PERMIT USE OF BIOREMEDIATION?** | Product must be on the NCP List to be considered for use. (National Oil and Hazardous Substances Pollution Contingency Plan, Product Schedule) |
| **3. DO HYDRODYNAMICS OF SPILL AREA ALLOW FOR AN EFFECTIVE USE OF BIOREMEDIATION?** | Any area that can be accessed through spraying or injecting of dispersants, can be accessed for Bioremediation. |
| **4. COMPARE CLEANUP ENDPOINTS AND TIMELINES** | **NA Type:** No defined endpoint and timelines<br>**MC Type:** No defined endpoint and timelines<br>**EA Type**: Defined endpoint: All hydrocarbons that have come in contact with EA Type converted to CO2 and water.<br>Defined Timeline: All hydrocarbons that have come in contact with EA Type, converted to endpoint in a few days to a couple of months. |
| **5. VERIFY BIOREMEDIATION AGENT IS ON THE NCP PRODUCT SCHEDULE** | By law, any product used for oil or hazardous substance spill cleanup on U.S. navigable waters must be on the NCP Product Schedule or it cannot be considered for use. |

| | |
|---|---|
| **6. MAP OUT IMPACTED AREAS AND DEFINE BIOREMEDIATION APPROACH** | Can area be accessed by land, boat, and/or air?<br>Work with manufacturer to define what amounts of product in what concentrations are appropriate to address each spill. |
| **7. IS THE NECESSARY INFRASTRUCTURE IN PLACE TO PERFORM THE REMEDIATION AND MONITOR RESULTS** | A) Does manufacturer have adequate stockpiles and production capabilities to immediately address the spill?<br><br>B) Can the necessary shipping, storage, and transport to spill site.be coordinated quickly?<br><br>C) Can the necessary equipment for product application be quickly obtained, if it is not already on site.<br><br>D) Set up before, during and after testing of relevant field samplings to ensure full removal of toxicity is achieved. |
| **8. OBTAIN RRT APPROVAL THROUGH SUBMISSION OF THE BIOREMEDIATION USE AUTHORIZATION FORM (in Appendix B)** | A completed authorization form should be transmitted to the RRT for the required authorization to proceed with bioremediation treatment. The RRT shall approve or disapprove the use of bioremediation within *24 hours* of receiving a completed form from an OSC. If the use of Bioremediation is denied, RRT must give written detailed reasons for denial of request. |
| **9. PROVIDE DETAILED WRITTEN RRT REQUEST DENIAL TO MANUFACTURER OF BIOREMEDIATION PRODUCT TO VERIFY THAT DENIAL IS BASED ON ACCURATE SCIENCE.** | If request to use Bioremediation is denied, ensure the denial is based on accurate information. If it is not, request immediate hearing in order to provide any further documentation necessary to address antiquated or inaccurate information that RRT decision makers may have. |

# BIOREMEDIATION FEASIBILITY ASSESSMENT CRITERIA

**IMPORTANT NOTE: THIS SECTION MUST BE BROKEN DOWN INTO TWO PARTS: ASSESSMENT CRITERIA RELATED TO 1) BIOREMEDIATION SUB-CATEGORY EA (ENZYME ADDITIVE), AND 2) BIOREMEDIATION SUB-CATEGORIES MC (MICROBIAL AGENTS) AND NA (NUTRIENT ADDITIVE).**

## 1) BIOREMEDIATION SUB-CATEGORY EA (ENZYME ADDITIVE)

Bioremediation Sub-Category EA is the only *first-response* bioremediation methodology. It is effective on fuels, fresh oil, medium weight oil, heavy oils (bunker C and ANS), and weathered heavy oils. See link: http://www.osei.us/pdf%20files/RRT%20plus%20testing.pdf. It can be utilized on the full scope of hydrocarbon potential as well as hydrocarbon-based hazardous chemicals including PCB's. The effectiveness of sub-category EA is not constrained by the varying characteristics of different types of oil. It can be utilized on gasoline/fuels if they pose an explosion hazard, in a populated area, port, or harbor, including oilrigs. Sub-category EA swiftly breaks the oil up into small particles, making it difficult to see. At the same time, it changes the density of oils, preventing them from sinking into the water column and thereby preventing the contamination of secondary, tertiary, and quaternary areas. This action prevents migration of the spill to more sensitive areas. The entire extent of the hydrocarbon material is expected to remediate 100%, especially the most persistent toxic components of the hydrocarbons in the PAH range, which has been demonstrated through testing and cleanup experience.

Sub-category EA can be used along with mechanical cleanup, if desired, however, mechanical cleanup is comparatively limited and costly. The DOI proved sub-category EA to be significantly superior in results (through performing test comparisons of OSE II, mechanical cleanup, and Corexits 9500 and 9527a). See link: http://osei.us/pdf%20files/OSEI-Summary-of-Department-of-Interior.pdf

**THE FOLLOWING SECTION DOES NOT APPLY TO BIOREMEDIATION FIRST RESPONSE METHODOLOGY. IT APPLIES ONLY TO BIOREMEDIATION SUB-CATEGORIES NA (NUTRIENT ADDITIVES) AND MC (MICROBIAL AGENTS), WHICH, DEPENDING ON VARIOUS CONDITIONS, CAN BE EFFECTIVE "POLISHING UP" PRODUCTS AFTER OIL SPILLS HAVE BECOME WEATHERED AND WHERE TOXICITY LEVELS HAVE BEEN SIGNIFICANTLY REDUCED. HOWEVER, A FULL FEASIBILITY STUDY WOULD NEED TO BE DONE ON ANY BIOREMEDIATION PRODUCTS UNDER THE MC AND NA SUB-CATEGORIES AS TO THEIR EFFECTIVENESS WITHIN A SPECIFIC GEOGRAPHIC AREA, TAKING INTO ACCOUNT CLIMATE, LOGISTICAL ACCESS TO THE SPILL, LONG-TERM ECOSYSTEM IMPACT, EFFICIENCY, NECESSARY REPETITION OF APPLICATION, ETC.**

## 2) BIOREMEDIATION SUB-CATEGORIES MC (MICROBIAL AGENTS) AND NA (NUTRIENT ADIDITIVES)

Assessing the feasibility of bioremediation sub-categories NA and MC is basically a two-stage process. The first stage determines whether a particular spill is a candidate for bioremediation NA or MC treatment. The second stage determines whether bioremediation with NA or MC can be implemented effectively, given the logistics of application and monitoring.

### Incident Characteristics

The characteristics of a spill incident provide indications of the extent to which bioremediation treatment with sub-categories NA or MC will be safe and effective against the contaminant spilled in a particular location. To aid in assessing the sub-categories NA or MC bioremediation as a response option in several different habitats, advisability information has been provided in the following sections. The matrix provides general guidelines regarding the advised use of bioremediation sub-categories NA and MC in different habitats based primarily on concerns for preserving habitats and minimizing harm to the indigenous flora and fauna.

### Characteristics of Spilled Oil

The possibility and practicality of applying bioremediation sub-categories MC and EA to the type of oil or petroleum product spilled must also be evaluated. That is, the extent to which the remaining chemical constituents of the spilled oil (which characterize that oil) are expected to be biodegradable needs to be assessed before “polishing up” bioremediation treatment with sub-categories NA and MC is considered further. Close to 100% of the entire extent of the hydrocarbon material should be expected to be remediated, per the Clean Water Act, especially the most persistent toxic components of the hydrocarbons in the PAH range. Biodegradation is typically useful on moderately to heavily oiled substrates, after other techniques have been used to remove as much oil as possible and on lightly oiled shorelines where other techniques are destructive or not effective. When used on diesel-type and medium oils that do not have large amounts of high molecular weight, slowly degrading components, bioremediation sub-category types NA and MC are most effective. On thick oil residues sub-category NA and MC are least effective. However, bioremediation with sub-category NA and MC agents should not be considered for gasoline spills, which can be swiftly removed with bioremediation sub-category EA type. Generally, oils can be divided into the following categories *(to further assist in making this determination see Appendix C, "Evaluating Biodegradation Potential of Various Oils")*.

Group I: Very Light Refined Products *(gasoline, naptha, solvents)*

- very volatile and highly flammable
- complete removal by evaporation likely or sub-category EA can be used to address any potential fire hazards

- high acute toxicity to biota
- can cause severe impacts to water-column and intertidal resources
- specific gravity less than 0.80
- will penetrate substrate, causing subsurface contamination

**not considered for bioremediation with sub-categories NA or MC due to high evaporation rates.**

Group II: Diesel-like Products and Light Crude Oils *(no.2 jet fuel oil, jet fuel, kerosene, marine diesel, West Texas Crude, Alberta Crude)*

- moderately volatile; persists in environment for an increasing period of time as weight of material increases
- light fractions will evaporate to no residue
- crude oils leave residue after evaporation
- moderate to high toxicity to biota
- can form stable emulsions
- tend to penetrate substrate; fresh spills are not adhesive
- specific gravity of 0.80-0.85; API gravity of 35-45
- **bioremediation with sub categories MC and NA is most effective on lower molecular weight oils, with faster degrading components; aromatic portions less susceptible to degradation**

Group III: Medium-grade Crude Oils and Intermediate Products *(North Slope crude, South Louisiana crude, no. 4 fuel oil, lube oils)*

- moderately volatile
- up to one third will evaporate in the first 24 hours
  moderate to high viscosity
- specific gravity of 0.85-0.95; API gravity of 17.5-35
- variable acute toxicity, depending on amount of light fraction
  can form stable emulsions
- variable substrate penetration and adhesion
- **bioremediation with sub-categories MC and NA is most effective on lower molecular weight oils, with faster degrading components**

Group IV: Heavy Crude Oils and Residual Products *(Venezuela crude, San Joaquin Valley crude, Bunker C, no. 6 fuel oil)*

- slightly volatile
- very little product loss by evaporation
- very viscous to semisolid; may become less viscous when warmed
- specific gravity of 0.95-1.00; API gravity of 10-17.5
- low acute toxicity relative to other oil types
- can form stable emulsions
- little substrate penetration; can be highly adhesive

**higher molecular weight and fewer number of straight-chained hydrocarbons makes bioremediation with sub-categories MC and NA less effective than on**

**medium oils**

Group V: Very Heavy Residual Products

- very similar to all properties of Group IV oils, except that the specific gravity of the oil is greater than 1.0 (API gravity less than 10). Thus, the oil has greater potential to sink when spilled.
- **higher molecular weight and fewer number of straight-chained hydrocarbons makes bioremediation with sub-categories MC and NA less effective than on medium oils**

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**Characteristics of Affected Habitats**

After evaluating the spilled oil's susceptibility to biodegradation, the habitats impacted by the spilled contaminant and the background level of nutrients in the impacted area should be identified and characterized. For each of the following habitats, the recommended approach is provided; *Optional*, **or** *Not Advisable*. [NOTE: "Not Advisable" does not preclude the OSC from conducting a Pilot Test to determine the effectiveness of bioremediation in an area. The harmful effects of the oil must be balanced against the potential effects of bioremediation.] The listed habitats are appropriate for marine, estuarine and riverine settings.

| | |
|---|---|
| Open Water:<br>sub-categories MC and NA: Not Advisable<br>sub-category EA: Optional | Off-shore Waters<br>sub-categories MC and NA: Not Advisable<br>sub-category EA: Optional |
| Tidal Inlets :<br>sub-categories MC and NA: Not Advisable<br>sub-category EA: Optional | Water Intakes<br>sub-categories MC and NA: Not Advisable<br>sub-category EA: if the water will go through treatment: Optional<br>Note: since sub-category EA causes oil to float, by booming off the intake area, sub-category EA will prevent the oil/hydrocarbons from sinking to the level of the intake, possibly protecting the intake |
| Small Lakes/Ponds:<br>sub-categories MC and NA: Not Advisable<br>sub-category EA: Optional | Small Rivers/Streams<br>sub-catgories MC and NA: Not Advisable<br>sub-category EA: Optional |
| Exposed Man-made Structures:<br>sub-categories MC and NA: Not Advisable<br>sub-category EA: Optional | Sheltered Man-made Structures<br>sub-categories MC and NA: Not Advisable<br>sub-category EA: Optional |
| Exposed Scarps in Clay | Wave-cut Clay Platforms |

| | |
|---|---|
| Open Water:<br>sub-categories MC and NA: Not Advisable<br>sub-category EA: Optional | Off-shore Waters<br>sub-categories MC and NA: Not Advisable<br>sub-category EA: Optional |
| sub-categories MC, NA and EA: Optional | sub-categories MC, NA, and EA: Optional |
| Fine-grained Sand Beaches<br>sub-categories MC, NA, and EA: Optional | Sandy Banks<br>sub-categories MC, NA, and EA: Optional |
| Mixed Sand and Shell Beaches<br>sub-categories MC, NA, and EA: Optional | Shell Beaches or Banks<br>sub-categories MC, NA, and EA: Optional |
| Exposed Rip-rap<br>sub-categories MC, NA, and EA: Optional | Sheltered Rip-rap<br>sub-categories MC, NA, and EA: Optional |
| Exposed Tidal Flats:<br>sub-categories MC and NA: Not Advisable<br>sub-category EA: Optional | Sheltered Tidal Flats:<br>sub- categories MC and NA: Not Advisable<br>sub-category EA: Optional |
| Salt to Brackish-water Marshes:<br>sub-category MC: Not Advisable for most<br>sub-categories NA and EA: Optional | Freshwater Marshes:<br>sub-categories MC, NA, and EA: Optional |
| Freshwater Swamps:<br>sub-categories MC, NA, and EA: Optional | Mangroves:<br>sub-categories MC, NA, and EA: Optional |

**Open Water, Off-shore, Tidal Inlets and Water Intakes**

Bioremediation sub-categories MC and NA are not effective for the time-frames of concern, relative to the potential of transport of the oil to areas where it could affect more sensitive resources. Thus, bioremediation treatment with sub-categories MC and NA is not advisable for these habitats or areas.

Bioremediation sub-category EA has constituents that cause these matrices of the product to adhere to hydrocarbon-based material so that, wherever a spilled hydrocarbon based material migrates, it will float and remediate to CO2 and water. Because sub-category EA causes oil to float, it allows booming operations to prevent oil from getting near to water intakes, keeping the oil on the surface out of the direct suction area of the intake. When applied to oil on the open water, off-shore, and tidal flats, it breaks down the oil's structure, reduces toxicity and adhesion properties, causes the oil to float so other areas of the water column and seabed are protected. If oil reaches the shoreline, it does not adhere to the rocky shoreline, marshes, or sandy beach. For tidal flats the oil lifts off the flat and remediates on the surface of the water away from the shoreline once the tide goes out.

**Small Ponds, Lakes, Rivers and Streams**

Bioremediation sub-categories MC and NA are not applicable for gasoline and light oils due to the fact that their rapid evaporation occurs faster than either MC or NA become effective. There is insufficient information on impacts and effectiveness for other oil types, however, paricularly with sub-category NA, there are special concerns about nutrient overloading in small, restricted water bodies.

Category EA can be used on fresh water. It lifts oil off shoreline grass and sandy areas, and living and dead marsh grass. It holds oil on the surface, where the enhanced indigenous bacteria spread on the oil's surface, while the bioremediation process is converting the oil to CO2 and water.

**Solid Man-Made Structures: Exposed and Sheltered**

Oiling of exposed sea walls usually occurs as a band at the high-tide line. This type of oiling is not amenable to bioremediation sub-categories MC and NA because of difficulty of application and low effectiveness.

Category EA can be used to address man-made vertical or horizontal structures, due to its ability to break down molecular structure of the oil and lift the hydrocarbons out of the pours of the concrete, stone, rock, or off of wooden structures.

**Exposed Scarps in Clay and Wave-Cut Clay Platforms**

Because of their erosional nature, removal of lightly oiled sediments may not be recommended on these habitats. Bioremediation subc-categories MC and NA may be an option whereby the oil could be treated in place.

Category EA lifts hydrocarbon-based material off sediment, preventing oil from blanketing the area. It's reduction of oil's adhesion properties can prevent re-oiling.

**Fine-grained Sand Beaches or Sandy Banks**

On outer beaches with low recreational use, bioremediation sub-categories MC and NA may be an option, particularly for light oiling or residual oil left after other countermeasures have been completed.

Sub-category EA is an option, when handling recreational or non-recreational beachs. During an oil spill incident, it is vital to rapidly break down the oil's molecular structure, reduce toxicity and break down adhesion properties to be able to swiftly remove the oil and remediate it to

CO2 and water. This protects the shoreline, the various life forms, and the public's health, and prevents re-oiling.

Fine-grained sand beaches also occur along bay margins and dredge spoil banks. Sandy banks occur along rivers. These habitats typically occur in more sheltered areas, where natural removal of residual oil by wave or current action will be slower then along exposed beaches. They are often not amenable to mechanical removal with sub-categories NA or MC, thus manual removal of heavy accumulations of oil or oiled wrack is sometimes attempted. Bioremediation with sub-categories MC and NA may be considered for sites with light oiling or residual oil left **after** manual removal efforts have been terminated.

Sub-category EA precludes the manual removal of oil since mechanical manual removal disrupts the intertidal zone environment, and human removal often needlessly exposes responders to toxic oil, and its gasses. Whether the beach is recreational or not, the oils molecular structure can be rapidly broken down and the adhesion properties reduced, thereby allowing the oil to be lifted off the sandy beach or shoreline through the natural hydraulic lift properties of sub-category EA. This removes the oil, and the remediation to CO2 and water follows. With reduced adhesion properties, re-oiling is prevented.

## Mixed Sand and Shell Beaches and Shell Beaches or Banks

For lightly or moderately oiled beaches and banks, particularly where mechanical cleanup may result in removal of large amounts of sediment or be logistically difficult, bioremediation with sub-categories MC and NA may be considered. This option is best considered for sites without significant recreational use.

Sub-category EA is an option whether the beach is recreational or not. The oil's molecular structure should be rapidly broken down and the adhesion properties reduced, thereby allowing the oil to be lifted off the sandy beach or shoreline through the natural hydraulic lift properties of sub-category EA. This removes the oil, and the remediation to CO2 and water follows. With reduced adhesion properties, re-oiling is prevented. Not taking action, which was a recommended option in an earlier version of this document, allows toxic hydrocarbons to impact the mixed sand, shell beach or banks and linger for a protracted period of time.

## Riprap: Exposed and Sheltered

Oil on riprap can occur as a coating on the boulders or as persistent accumulations of oil in the void spaces between the boulders. Neither type of oil is amenable to effective removal by bioremediation sub-category NA or MC techniques, under most conditions. If products under either of these categories are being considered for use in handling oil on riprap.

Sub-category EA breaks down the molecular structure, reducing adhesion properties, resulting in oil lifting off riprap and out of the crevices, allowing it to remediate to CO2 and water. Because the adhesion properties are reduced, re-oiling is prevented. If tidal flow is not capable of carrying the oil out of crevices and protected areas, the oil will remediate to CO2 and water in place.

**Exposed Tidal Flats and Sheltered Tidal Flats**

Both of these habitats are inundated daily by high tides, which result in rapid dilution and flushing of applied nutrients. Bioremediation sub-categories MC and NA are not likely to be effective under these conditions. There are significant toxicity concerns for use of bioremediation agents in shallow, poorly flushed areas, such as sheltered tidal flats, or subtital habitats where there are concentrations of sensitive life stages of fish and shellfish, such as sea grass beds and oyster reefs.

Sub-category EA is acceptable for high tides and flushing. Dilution is not a factor. Because the sub-category EA process includes molecularly attaching to the detoxified, broken down molecular hydrocarbon structure, and then causing the oil to lift and float, wherever the detoxified oil migrates to, it will still remediate to CO2 and water. Category EA's ability to detoxify the oil, reduce its adhesion properties and cause it to float in poorly flushed areas allows for separation from life stages of fish, shellfish, and sea grass beds, and allows the oil to be flushed out of the area with any minimal tidal movement. This can protect the living organisms in these areas not effected by the initial invasion of the oil to the area. Any oil trapped where sub-category EA has been applied will float and remediate to CO2 and water, preventing the lingering toxic effects of the oil in the area for an extended period of time.

**Salt to Brackish-water Marshes, Freshwater Marshes, Freshwater Swamps and Mangroves**

There are very few cleanup options that do not cause significant negative impacts to these sensitive habitats. However, there may be conditions under which bioremediation sub-categories MC and NA may be considered, particularly for lighter oils. In wetlands with shallow, poorly

mixed water bodies, the potential increase in eutrophication and ammonia caused by aggressive bioremediation with sub-categories MC and NA needs to be considered.

Sub-category EA when compared to past methods, and understandings, does not cause significant impacts to these areas, since the application is by spray apparatus. Once the area has been invaded by oil, the toxicity of the oil will be present and negatively impacting the ecosystem. Timely reduction in toxicity and removal of the oil is needed to safeguard these ecosystems. Sub-category EA's ability to breakdown the molecular structure by reducing the oil into small particles limits the toxicity, and can prevent the choking of the organisms by preventing the blanketing of the oil, reducing the adhesion properties, lifting the oil to the water's surface, allowing separation from vegetation or natural landscapes lessening the time the toxic oil can effect these areas. Sub-category EA causes any molecular weight oil to breakdown and lift up, even in poorly mixed areas, preventing eutrophication. Doing nothing, as was recommended in earlier versions of this document, unnecessarily allows for eutrophication to occur for an extended time and allows for the development of amonia. Sub-category EA limits the time and impact of the oil in the surrounding area and will not exacerbate the development of ammonia. Sub-category EA is mixed with the water from the nearby area so whether the area has fresh, salt, or brackish water the natural bacteria are enhanced and are already acclimated to the effected environment. Sub-category EA limits the time and amount of toxicity of the oil to the environment and allows for the oil to be separated from the flora and fauna of the area.

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## LOGISTICAL CONCERNS

Characteristics of a spill incident, including characteristics of affected habitats and spilled hydrocarbon based pollutants, should determine whether a spill is a candidate for bioremediation treatment. As Bioremediation sub-category EA (Enzyme Additive) can be utilized in almost all scenarios as a first-response methodology that has no limited window of opportunity (such as chemical dispersants), does not introduce further toxicity in the environment, and rapidly converts the spilled hydrocarbons to CO2 and water without necessitating secondary, tertiary and quaternary cleanup. Because its methodology most closely achieves the mandates of the Clean Water Act, it should be considered as the primary potential methodology for employment. Once bioremediation has been chosen as the method of response, then the logistical feasibility of implementing an appropriate bioremediation action plan should be evaluated. Implementation considerations include the proposed scale of a bioremediation activity, the availability of the bioremediation agent(s) proposed for application, and the availability of the resources necessary to conduct the application and monitoring recommended for the agent(s) proposed for use in each affected habitat.

### Scale of Bioremediation Response

The first step in assessing the logistical feasibility of bioremediation is to determine the scale of the bioremediation response. The scale of the bioremediation response refers to the extent to which bioremediation will be involved in the cleanup, particularly in terms of the size of the area. The scale of the bioremediation response effort will determine the amount of agent(s), the number of personnel, and the equipment resources necessary to complete the chosen treatment technique and monitoring of the bioremediation response effort.

### Agent Availability

Once the proposed scale of the bioremediation response activity has been determined and agent alternatives have been identified, the availability of these agents for use at the spill location should be assessed. If an agent is not available in quantities necessary to complete the bioremediation response activities, the scale of the bioremediation response should be reevaluated, a different bioremediation technique should be considered, or bioremediation should be eliminated as a response alternative. Whereas in the past, large stockpiles of Bioremediation agents were impossible to come by, this factor is no longer a problem, particularly as it relates to Bioremediation sub-category EA type.

### Application and Monitoring Resources

Several application methods are generally available for bioremediation agents and each method may have unique resource requirements for its implementation. To determine whether requirements for application methods will preclude or limit the use of a particular method, the habitat(s) where bioremediation is being considered for cleanup should be evaluated to determine which method is most appropriate.

Because it attaches itself to the oil, sub-category EA can be deployed by any spray apparatus as well as from eductor or induction systems, and can be deployed in the same manner by aerial spray with a slight adjustment in the pre mixing in the craft with water. Most Contractors that can be deployed for dispersant application with a minor adjustment can apply category EA. Vessels of opportunity can be outfitted easily to apply sub-category EA and most commercial vessels in ports contain some type of induction fire fighting systems that can be utilized for sub-category EA application.

Next, the types and supply of available equipment and personnel adequate to implement and monitor the bioremediation response effort, as well as access to laboratory facilities for sample analyses, should be evaluated. (Refer to the Biomonitoring Plan section for recommended monitoring activities and monitoring resource requirements.) If the desired bioremediation response requires more resources than are currently available or attainable, the scale of the bioremediation response may need to be reduced. Similar to dispersants, biomonitoring with sub-

category EA can be visually observed to discern the oil reactions to the remediation process. Dissimilar to dispersants, the observers will not be at risk of exposure to toxic chemical components.

## IMPLEMENTATION

Before initiating bioremediation treatment, several steps shall be completed. First, the OSC shall notify RRT 4 that the use of bioremediation is being proposed by transmitting the completed Bioremediation Use Authorization Form. Second, a Bioremediation Work Plan and Bioremediation Monitoring Plan shall be developed to address issues necessary to ensure an efficient and effective bioremediation spill response. These plans can and should be pre developed before a spill emergency so there is no guess work when a spill event occurs. This document covers most of the spill scenarios, and a sample work plan that is appended to this document is filled out so that responders can be prepared to respond to the different spill scenarios noted in this document.

### RRT Notification

After finalizing the selection of a bioremediation treatment technique and the appropriate method for each affected habitat to receive treatment, the completed Bioremediation Use Authorization Form shall be transmitted to the affected State(s), EPA Region 4, the appropriate USCG District and the Federal Trustees for concurrence and consultation with the decision. If applicable, the appropriate Federal Land Manager (e.g., DOI) should also be notified.

If use of bioremediation in the spill area has been pre-approved or pre-authorized by RRT 4, this concurrence is not necessary. However, the OSC must still notify RRT 4 of the decision to use bioremediation. In the event RRT 4 pre-authorizes an area for the use of bioremediation, such areas will be included in the plan by addendum. In order for contractors to be able to swiftly respond to a spill to prevent as much potential damage as possible to the environment, marine life, wildlife, and human health, pre-approval of Bioremediation sub-category Enzyme Additive (EA) should be initiated, so that first-response, non-toxic bioremediation can immediately be carried out

## BIOREMEDIATION WORK PLAN (ATTACHED SEPARATELY)

Work plans are important to ensure the safe, coordinated, and well documented implementation of bioremediation. Work plans are comprised of systematic procedures and guidelines that clarify and resolve issues such as worker and public safety, documentation requirements, response personnel roles and responsibilities, treatment technique agent application protocols, and application control and oversight considerations. Complete Work plans must include spill and site specific considerations. It is essential in a response that every incident or event be managed according to a plan and bioremediation is no exception. The Work plan shall provide:

- A clear statement of objectives and actions.
- A basis for-measuring work effectiveness and cost effectiveness.
- A basis for measuring work progress and for providing accountability.

Plans should be prepared for specific time periods or operational periods. These periods can be of various segments of time. Decisions on the length of the operational period or time segments may be affected by the length of time available/needed to achieve objectives, the availability of resources, environmental considerations, and safety considerations. Essential parts of any Work plan are:

1. **Statement of objectives -** Statement of what is expected to be achieved. Objectives must be measurable.

2. **Organization -** Describes what organization will be in place. This will describe in detail the specific roles and responsibilities of the participants in a bioremediation treatment technique. This will also describe the interaction of one entity to another.

3. **Tactics and assignments -** Describes tactics and control operations and what resources will be assigned. If the application is a large one, resource assignments may be done by groups.

4. **Supporting material -** Examples include a map or sketch of the area(s) to be treated, communications, traffic plan, weather data, special precautions, and safety information.

All supervisory personnel must be familiar with the plan and any changes which develop throughout the life of the project. This can be accomplished through briefings and by distributing copies of the written plan.

The Work plan must include an avenue to provide for ongoing evaluation of the plan's effectiveness. Supervisors should regularly assess work progress against control operations called for in the plan. If deficiencies are found, improved direction or additional staffing may be required, tactical operations may need to be modified, and/or changes may need to be reflected in planning for the next segment of time.

Demobilization activities, although often overlooked, are an integral part of the

Work plan. As the project begins to wind down, everyone will be anxious to leave the scene and return home. Demobilization planning helps to assure a controlled, safe, efficient, and cost effective demobilization process.

### Organization

The response structure or organizational framework identifies the participants in a response, their general areas of responsibility, and the lines of authority among them. A chart illustrating the participants in a bioremediation response activity in Region 4 and their inter-relationships would be very helpful in summarizing this information. In developing this section, the following questions should be addressed:

- Who will manage the overall bioremediation activity?
- Who will be the likely participants (e.g. federal and state agencies) in the activity for the Region? What are the general roles?
- Who will be the likely participants, if any, from outside the Region? What are the general roles?
- Who will manage the monitoring portions of the activity?
- Who will develop an appropriate Work plan for the bioremediation activity?
- Who will perform specific treatment method or agent(s) application(s)?
- Who will perform monitoring?
- Who will perform public outreach?

Describe in detail the specific roles and responsibilities of the likely participants (RRT, federal and state agencies, international governments/agencies, non-governmental organizations, responsible parties, etc.) in a bioremediation activity in Region 4. The information in this section should coincide with the information presented above on the regional response structure.

### Tactics and assignments

Tactical direction includes determining the tactics and operations necessary for the selected strategy and determining and assigning the appropriate resources.

Resource assignments should be made for each specific work task. Such assignments should consists of the kind, types and numbers of resources available and needed to achieve the desired outcomes.

Personnel and logistical support factors must be considered in determining tactical operations. Lack of logistical support can mean the difference between success and failure in achieving objectives.

**Supporting Material**

Public Safety/Information - Public safety is paramount in any bioremediation project. The following are some suggested actions which should be taken during a spill response to ensure public awareness and protection:

> Provide news releases and updates to newspapers, radio, television stations, and neighboring areas that could potentially be impacted by bioremediation activities. Be prepared to discuss details regarding the chosen treatment technique in simple layterms so the affected public will have an understanding of exactly what to expect and what the expected benefits are.

Site/Worker Safety - Worker health and safety is always the foremost concern during any spill response action. Since all oil spill response actions require a health and safety plan and the bioremediation application is merely a facet of the total spill response effort, the existing heath and safety plan should be used for the bioremediation application and augmented with the specific safety hazards associated with the bioremediation treatment method or agent application. A section referred to as biological hazards should be included in all health and safety plans associated with oil spill responses where biological agents are used as a response tool. This section should discuss the specific health and safety concerns associated with possible exposure to biological agents and include material safety data sheets (MSDS) for all agents being used. At a minimum, the health and safety plan should address the following aspects of the bioremediation treatment method/monitoring program:

1. minimum health and safety concerns,
2. potential hazards during application and monitoring,
3. evaluations of those identified hazards,
4. actions described to minimize the potential hazards, and
5. response(s) needed if hazard does effect worker(s).

The following documents contain guidance on the preparation of health and safety plans:

1. OSHA 1910.120 and EPA 40 CFR 311,
2. USEPA, OERR ERT Standard Operating Procedures,
3. NIOSH/OSHA/USCG/EPA Occupational Health and Safety Guidelines,
4. ACGIH Threshold Limit Values, and
5. existing local and area contingency plans.

To avoid disturbances to the treated area after treatment, all treated and control sites should be secured by the best achievable means. To avoid possible injury, post

warning signs or secure the treated area to differentiate the site from surrounding localities.

## BIOMONITORING PLAN

Bioremediation is assumed to enhance the biodegradation of oil or hazardous substances without increasing adverse impacts to human or ecological health. As there is now defensible documentation from over 18 toxicity tests on fresh and salt water species, (many performed by the EPA or under EPA contract), and voluminous field use to confirm this assumption as it relates to Bioremediation sub-category EA, non-toxic bioremediation should be considered the primary first-response methodology considered. Where applicable, the use of bioremediation sub-categories MC and NA effectiveness and safety need to be monitored through a sound program of applied science. 18 toxicity tests can be viewed and downloaded at this link: http://osei.us/wp-content/uploads/18-Toxicity-test-with-4-2012-Log0.pdf

As with any response methods, (dispersants, skimming, in-situ burning, etc.) an associated monitoring program should be conducted when bioremediation treatment (either natural or enhanced) is used as a response tool. The plan outlining the biomonitoring program will be referred to as the biomonitoring plan.

### Objectives

The principal objectives of the monitoring program and the elements of each objective are listed below.

1. Determine the efficacy of the selected bioremediation treatment method as it relates to the degradation of the spilled material.

   To continue to use biological degradation, the response community must compile data which shows that the use of bioremediation accelerates the breakdown of oil in the environment at a faster rate than if the oil was left to breakdown and degrade naturally. If there is no proven acceleration of the breakdown, then the risks and costs associated with the use of biological methods may outweigh the advantages.

2. Measure the environmental impact, if any, resulting from the biotreatment of an area, throughout the response activity to ensure against the harmful effects from the response. Especially, monitor any increases in eutrophication or ammonia caused by bioremediation.

   The monitoring of water quality parameters throughout the bioapplication is essential due to, in the case of Bioremediation sub-categories MC and NA, the potential for algae blooms, dissolved oxygen depletions, elevated available toxins in the water column, all of which may result in a critical impact to aquatic and vegetative life.

3. Determine if the bioremediation end points have been reached.

With the use of all response tools it is important to determine at what point the tool is no longer effective or at what point it has achieved its objective. Thus biomonitoring end points must be developed for Bioremediation Sub-categories MC and NA prior to the initiation of the application, keeping in mind these end points may need to be modified as the program progresses. Bioremediation Sub-category EA already has an established specific, defined endpoint: once it has come in contact with the oil, 100% of the hydrocarbons converted to CO2 and water within a few days to 2-3 months

4. Ensure the comparability of data collected from all bioremediation response efforts conducted within Region 4 through compliance with USEPA Region IV=s Sampling Standard Operating Procedures.

This is done in order that the data may be used to enhance our understanding of bioremediation as an oil spill response tool. Properly collected, validated and interpreted data will provide critical information to assess the efficacy and environmental impact of bioremediation treatment and related response activities. Such documentation is needed to identify and correct problems in the biological treatment process, to determine whether bioremediation endpoints have been reached, to ensure that bio treatment is less environmentally harmful than the spilled pollutant and to support cost recovery and other legal actions.

Secondarily, the data can be used for developing regional and national data bases, interfacing with natural resource trustees, preparing interim and final reports, and revising this biomonitoring plan.

**Quality Assurance**

The quality of environmental data used to support OSC decision-making is critical to a spill response that considers or uses bioremediation. The primary goal of the quality assurance (QA) program is to ensure the accuracy of the environmental data considered by the OSC and RRT 4. It is the QA policy of RRT 4 that all activities associated with data collection and derivation are to be documented thoroughly. A monitoring program manager should be selected to specify procedures for ensuring the quality of data generated through the monitoring program and for providing sufficient resources for QA of collected data.

**Biomonitoring Plan Design**

Each biomonitoring program, in large part, will be event/site specific; however, pre-event planning and standardization of collection/analysis methods is encouraged. The design of the biomonitoring program is two-fold: (1) to document any impact to water quality which might result from the treatment or application and (2) to provide for

the evaluation of the effectiveness of the treatment method or applied agent(s).

Conducting biomonitoring does not preclude the OSC/RP from conducting any other required monitoring associated with the spill event.

Project planning and site reconnaissance are essential activities conducted prior to the design of the biomonitoring plan. The OSC/RP may wish to refer to the area contingency plan (ACP) for existing shoreline or site assessment procedures developed by the area committees. The purpose of site reconnaissance activities are to gather information sufficient to:

- Determine that the objectives of the biomonitoring plan are consistent with the features of the site selected for application;
- Identify the type and quantity of existing historical water quality data for the area selected for the application, such as nutrient loading trends and physical water parameters;
- Define the geographic area of the spill targeted for application, for physical and chemical characteristics important to the design and execution of the biomonitoring plan;
- Determine the distribution, abundance, and seasonality of habitats, in the area to be considered for application;
- Project weather forecasts, meteorological and hydrogeological trends in the potential application area, for the proposed application time period;
- Determine equipment needs based on operational logistics; and
- Develop procedures designed to document sample collection methods and procedures.

The extent of the biomonitoring program should be directly proportional to the complexity and sensitivity of the area(s) chosen for biological degradation. The more diverse and sensitive the effected environment, the more complex and extensive the biomonitoring program should be. The volume of material spilled is not the driving factor in determining the extensiveness of the biomonitoring program; however, the larger the spill, in general, the more area affected and the greater the potential for affecting sensitive ecosystems. Thus, large spills generally will require a more extensive biomonitoring program. The OSC/RP should refer to the ACP and incorporate any and all required monitoring as directed by the ACP.

Because one spill event may affect several different morphological environments or habitats, bioremediation treatment techniques may be applied in several different habitats. The supporting biomonitoring program must be designed to accommodate inherent differences which are present in each habitat. Thus, each discrete habitat, within an application area, may require its own monitoring program.

**Monitoring Activities**

Biomonitoring plans should ensure that observations and samples be collected and analyzed from the following areas - within each discrete habitat(s):

**Untreated areas**

1. uncontaminated, untreated source areas (this will serve as background information and may not require the same intensity of sampling as the other areas),
2. contaminated, untreated source areas, and

**Treated areas**

3. contaminated, treated areas

In order to evaluate the effectiveness of the bioremediation treatment technique, the biomonitoring plan should provide for the comparison of replicate data from treated and untreated areas for the duration of a project.

Within each discrete habitat which is a part of the bio application project, treated and untreated sites that exhibit similar chemical and physical characteristics should be chosen. Their similarity will support the comparability of the data generated. During their selection the following criteria should be considered, (1) environmental parameters, (2) physical habitat and geomorphology, and (3) oil loading and the probability of further oiling. Site variability should be limited as much as possible in order to generate data which is comparable.

Other physical variances which may effect the integrity of the data collected are wave action, tidal flushing, currents, boat traffic, and exposure to wind or other external forces.

Because efficacy analyses focus on evaluating relative changes in the concentration of the constituents of oil between treated and untreated sites, it is important to ensure that uncontaminated source areas remain uncontaminated for the duration of the monitoring program and contaminated areas are not re-oiled for the duration of the monitoring program.

Monitoring should take in place in two forms:

1. Qualitative - serves as real time feedback for response decision and is usually in the form of visual observations, supported by photo documentation.

2. Quantitative - serves as the basis for longer term analysis of the success of the project and is in the form of sample collection and analysis.

Although visual observation is considered subjective, there is no substitute for this type of "real time" or fast feedback. Observers must be assigned to the project and trained to monitor morphological changes, which may occur to the oil as it breaks down and any changes in organism behavior, such as the occurrence of algae blooms and

fish kills.

All sample collection and analysis begins with a sampling plan. The sampling plans should include, at a minimum, the following:

- Implementation schedule (monitoring should be expected to take place over 3-4 months or until end points are reached)
- List of objectives
- Tasks to be conducted
- Description of project management
- Identification of sensitive areas included in/adjacent to the sample location areas
- Identification of sample locations, frequency, and collection methods
- Description of sample chain of custody procedures and QA/QC procedures
- Description of water quality history (if available) of the affected area or procedure for determining background values for the affected area if historical data does not exist.

The environmental characteristics and measurements that should be assessed and the samples that should be taken as part of the biomonitoring are presented in Table 2, along with a schedule for performing these activities. Sampling at each site, water depth (as appropriate), and time, should be performed in *duplicate for 10% of the samples collected*. Although the mix of samples collected should be based on the requirements of the analytical methods, minimum sample sizes are recommended as 1 liter for water samples and 4 - 16 oz for sediment or shoreline materials. All samples should be placed in precleaned jars or bottles with Teflon lined caps, as appropriate.

The monitoring parameters should involve a tiered approach which utilizes relatively inexpensive techniques such as total petroleum hydrocarbons (TPH) for screening and more sophisticated methods that target individual petroleum constituents to confirm biodegradation efficacy in *at least 25% of the sample*s *analyzed*. The latter would include GC/MS analysis of target aliphatic and aromatic hydrocarbons which have been identified as marker compounds for tracking oil degradation and weathering, such as the normal alkanes, the isoprenoids, pristane and phytane, and the conservative biomarker hopane. Water quality measurements should include nutrients, dissolved oxygen, biological oxygen demand (BOD), TOC and COD. Refer to Appendix E for methodologies and recommended procedures.

All data is subject to review by the OSC or a delegate and will be made available upon request. This data will support further response decisions and to provide the response community with a better understanding about the use of bioremediation as an oil spill response tool.

## DOCUMENTATION AND REPORTING

During the course of a bioremediation activity and accompanying monitoring effort, the following reports shall be prepared and submitted to the OSC:

Activity reports -- provide descriptions of the bioremediation activity area, weather, unique observations, and activities undertaken, as well as the names and affiliations of persons on site. Activity reports should be prepared whenever activities on a site are undertaken.

Analytical reports -- provide laboratory analysis results of environmental and control samples. Lab results should be analyzed, interpreted and a brief summary report prepared within a reasonable time agreed to by all parties.

After action report -- provide a description of the overall bioremediation activity and accompanying monitoring effort, including results of both field and laboratory activities. A draft should be submitted within 30 days after the end of the monitoring effort. A final report, (incorporating comments from those the draft was submitted to, as well as photos) should be submitted within 60 days after submission of the draft.

In addition, at the time the final after action report is submitted, all field notes, including those of contractors, should be submitted to the OSC.

To facilitate information transfer and the development of a data base on bioremediation use and bioremediation agents, the Bioremediation Use Follow-Up Form in Appendix F should be completed at the end of the bioremediation activity.

**PLAN REVISION**

The monitoring plan and suggested procedures outlined in this section should be implemented and modified, as necessary, based on the cumulative experience and knowledge gained from conducting bioremediation field activities and associated laboratory activities. Recommendations for revisions should be submitted to the Region 4 RRT for approval.

**TABLE 1 – FIELD-MONITORING PARAMETERS**

| Parameter | Sample Size[1] | Assessment/ Collection Location | Assessment/ Collection Frequency[2] |
|---|---|---|---|
| Visual observations (mortality, behavioral effects, appearance changes, oil distribution) | N/A | All test sites | Daily to the extent possible; at least each day that water, sediment, and/or shoreline material sampling is performed |
| Temperature (air, water) | N/A | All test sites | Days 0, 1, 7, 14 and every week thereafter |
| Salinity | N/A | All test sites | Days 0, 1, 7, 14 and every week thereafter |
| Dissolved oxygen | N/A | All test sites | Days 0, 1, 7, 14 and every week thereafter |
| Sea state | N/A | Activity area | Days 0, 1, 7, 14 and every week thereafter |
| Current | N/A | Activity area | Days 0, 1, 7, 10 and 20 |
| Wind velocity | N/A | Activity area | Days 0, 1, 7, 14 and every week thereafter |
| Efficacy (water, sediment, and/or shoreline material) | 1 liter water; 20 grams sediment or shoreline material | All test sites and, as appropriate, all water depths | Days 0, 1, 7, 14 and every week thereafter |
| Toxicity[3] (water, sediment, and/or shoreline material) | 8 liters water; 20 grams sediment or shoreline material | All test sites and, as appropriate, all water depths | Days 0, 1, 7 for Microtox and at same intervals for every reapplication of agent, for long term amphipod days, 0, 1, 7, 14 and every week thereafter |

[1]N/A means "Not Applicable".
[2]Frequency is relative to the time of agent application.
[3]Sample size, location and frequency for toxicity testing are recommendations.

Actual parameters shall be determined based upon conditions of the spill event.

# APPENDIX A

## APPLICABLE FEDERAL AND STATE REGULATIONS

Legislation at both the federal and state level may affect decisions to use bioremediation. Existing regulations and policies that govern the use of bioremediation treatment techniques and agents in responses to spills in Region 4 are summarized below.

### Federal Regulations

At the Federal level, Subpart J of the NCP governs the use of chemical and biological agents -- which include bioremediation agents -- in responding to oil spills. Specifically, the Subpart:

Restricts the use of chemical and biological agents that may affect US waters to those listed on the NCP Product Schedule;

Specifies technical product information that must be submitted to EPA for an agent to be added to the Schedule; and

Establishes conditions for obtaining authorization to use chemical or biological agents in a response action.

If EPA determines that the required data were submitted, EPA will add the agent to the Schedule. Note, however, that listing of an agent on the NCP Product Schedule does not constitute approval of that agent for use or confirmation of any claims regarding the agent's safety or effectiveness.

Data on agents listed on the NCP Product Schedule are available through EPA's Emergency Response Division in Washington, DC.

The OSC, with concurrence of RRT 4, including the RRT representative from the State with jurisdiction over the waters threatened by the spill, may authorize the use of any agent listed on the Product Schedule. In addition, when practicable, the OSC should consult with the Department of Commerce (DOC) and Department of Interior (DOI) representatives to the RRT before making a decision to bioremediate a spill. If the use of particular products under certain specified circumstances is approved in advance by the State, DOC, and DOI representatives to the RRT, **and** such preapproval is specified in the Regional Contingency Plan, the OSC may authorize bioremediation without consulting the RRT.

## State Regulations and Policies

The following States do not currently have set policies regarding the use of bioremediation during a spill event. For approval or information, contact the State=s representative to the Region 4 RRT.

| | |
|---|---|
| **Alabama** | **Georgia** |
| **Kentucky** | **Mississippi** |
| **South Carolina** | **Tennessee** |

### Regulations and Policies in the State of Florida

The State of Florida does not have any regulations that specifically address the use of bioremediation as a spill response tool. However, regulations do specify that any person discharging a pollutant shall immediately undertake actions to contain, remove, and abate the discharge (Chapter 376.305(l), Florida Statutes) to the satisfaction of the Department of Environmental Protection (DEP). The DEP does not encourage bioremediation as a primary response countermeasure, but instead it may be used in conjunction with other conventional remedial actions. The exception to this is when the option of doing nothing is considered or conventional cleanup/treatment methods are not feasible. In those cases, in-situ bioremediation can be an effective substitute for traditional cleanup technologies. The State DEP has, however, approved bioremediation products for use in their state.

The DEP has developed a set of guidelines to assist the state OSC or first responder with bioremediation decisions and proper use. The *Guidelines for the Use of Bioremediation as a Cleanup Technique* apply to spills of less than 50 gallons of petroleum on inland areas or in non-navigable waters of the state. The DEP has not established any guidelines or policies regarding the use of bioremediation for coastal spill response. In these cases, the DEP will work closely with the Florida Marine Research Institute, the federal OSC and the RRT to identify areas where bioremediation would be considered.

The use of bioremediation is prohibited for petroleum contaminated site (inland UST sites) remedial actions unless specifically approved by the DEP Bureau of Waste Cleanup, Technical Support Section. The DEP has established petroleum contaminated soil cleanup criteria (Chapter 62-770, Florida Administrative Code) and publishes *Guidelines for the Assessment and Remediation of Petroleum Contaminated Soil* to clarify the DEP's position concerning petroleum contaminated soil remedial actions.

### Regulations and Policies in the State of North Carolina

The State of North Carolina's Department of Environment, Health, and Natural Resources regulates the use of bioremediation for response to spills. When requesting an

evaluation to utilize bioremediation the following information must be submitted to:

Dr. Luanne Williams
North Carolina Department of Environmental, Health and Natural Resources
Occupational and Environmental Epidemiology Section
PO Box 29601
Raleigh, NC 27626-0601
(919) 715-6429

**Required General Information**

1. Division of Environmental Management (DEM) contact person and phone number.
2. Current or future use of site with site contact person, address & phone number.
3. Contractor applying product, contact person, address & phone number.
4. Distance and impact to public or private wells used for drinking, industrial processes, cooling, agriculture, etc. and is area served by public water supply? Verification must be provided by the regional Groundwater and Public Water Supply Sections. Send responses to Dr. Luanne Williams.
5. Detailed specifications of the contamination present in the soil and/or groundwater.
6. Approximate distance & name of nearest surface water body (provide map).

**Required Product/Process-Specific Information** (All information submitted will be maintained as proprietary and not disclosed to other parties.)

1. Product manufacturer name, address, phone number and contact person.
2. Genus/species/strain of microorganism(s) contained in product
3. Identity of specific ingredients and concentrations of ingredients contained in the product and purpose of each.
4. Documentation of evidence from authoritative technical references (i.e. Bergey's Manual of Systematic Bacteriology, Bergey's Manual of Determinative Bacteriology or other existing references) that the microorganism(s) are not pathogenic to animals or humans.
5. Documentation (i.e. references) of whether or not the microorganism(s) are naturally occurring in the immediate or similar environment.
6. Documentation (i.e. references) of specific degradation products expected.
7. Documentation (if available) of migratory potential of microorganisms and degradation products in soil and groundwater.
8. Complete description of the bioremediation process on a site (e.g. application of the product to soil and/or groundwater, aeration of soil, procedures needed to maintain growth and chemical degradation).

The risk evaluation will be forwarded to the designated contact person within the company, site owner, manufacturer, consultant applying the product, DEM contact person and Groundwater Section contacts--Linda Blalock (Federal Trust Fund) and Brian Wagner (Operations Branch).

**APPENDIX B**

**THIS *BIOREMEDIATION USE AUTHORIZATION FORM***
**HAS BEEN FILLED OUT AS IT WOULD BE FOR BIOREMEDIATION**
**SUB-CATEGORY EA TYPE – OSE II**

## BIOREMEDIATION USE AUTHORIZATION FORM

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The following questions should be answered, if known, and presented to the OSC who will review them and present them to the RRT for consideration. A question left unanswered will not automatically result in a no-go decision, but EVERY effort should be made to present accurate and timely information. The RRT will use the information provided below to assist in making the decision for use of bioremediation.

The form consists of two parts, incident characteristics and feasibility assessment criteria. Additionally, a Bioremediation Work plan and Biomonitoring Plan must be prepared and submitted to the OSC or his designee for review. (Note: Many of the items requested in the feasibility assessment criteria section can and should be included in the bioremediation Work plan.)

### Incident Characteristics

Time and date of release: (TO BE FILLED IN WITH SPECIFICS OF INCIDENT)

Product spilled: (this is not a determining factor regarding the use of OSE II since sub-category EA/ OSE II handles all hydrocarbon-based materials. However, the product spilled should be noted.

Quantity spilled: (TO BE FILLED IN WITH SPECIFICS OF INCIDENT)

Status of spill: whether the oil is fresh or fully weathered, or is a continuing leak, or one that has been contained, sub-category EA/ OSE II can be applied effectively at any point.

Location of incident: (TO BE FILLED IN WITH SPECIFICS OF INCIDENT – SUB-CATEGORY EA/OSEII CAN BE USED IN ANY LOCATION)

Description of incident: (TO BE FILLED IN WITH SPECIFICS OF INCIDENT)

Properties of spilled product: THIS TYPE OF OIL SPILL DOES NOT IMPACT THE EFFECTIVENESS OF OSE II – OSE II WORKS ON ANY TYPE OF HYDROCARBON-BASED SPILLED PRODUCT

specific or API gravity
viscosity, cp
pour point, at temp, F
sulfur content, %w

Responsible party information: (to be filled in with specifics of incident)

company
address
telephone
contact person
telephone

**Feasibility Assessment Criteria**

Specific location proposed for treatment: SUB-CATEGORY EA CAN BE USED IN ALL OF THE CATEGORIES LISTED BELOW

- What are the characteristics of the spill environment?
- type of environment, habitat
- marine, brackish, freshwater
- past spill history

Amount of weathering spilled product has undergone: THIS IS NOT RELEVANT WITH SUB CATEGORY EA / OSE II – OSE II CAN BE UTILIZED ON FRESH, LIGHT, HEAVY OIL, AND WEATHERED OIL/ HYDROCARBON-BASED MATERIAL EQUALLY EFFECTIVELY

Description of impact(s):

- Has ownership of land been determined: (TO BE FILLED IN)

- Has written permission from landowner been obtained: (TO BE FILLED IN)

- Bioremediation agent proposed for use: SUB-CATEGORY ENZYME ADDITIVE
- Name of product. OSE II
- Type of agent (microbial, nutrient, microbial + nutrient, etc.). ENZYME ADDITIVE
- Is agent listed on NCP? YES. It is B-53 ON THE NCP LIST PRODUCT SCHEDULE UNDER CATEGORY BIOREMEDIATION, SUB-CATEGORY ENZYME ADDITIVE TYPE (EA)
- Has EPA data been reviewed by the SSC? YES
- To what tier has the agent been formally evaluated? TIER III
- Does the agent or responsible party have any previous first hand experience with the use of the proposed bioremediation agent, or have any corroborated (laboratory or field) data indicating it enhances biodegradation and is not toxic to affected spill environment? YES – EPA HAS SUFFICIENTLY TESTED IT FOR TOXICITY AND PROVEN IT TO BE COMPLETELY NON-TOXIC. THE EPA HAS SUFFICIENTLY TESTED IT FOR EFFICACY AND PROVEN IT WORKS ON WEATHERED OIL AS WELL AS HEAVY AND EMULSION OIL. THE DOI HAS SUFFICIENTLY TESTED IT FOR EFFICACY AND PROVEN IT IS MORE EFFECTIVE THAN DISPERSANTS AND MECHANICAL. DOE HAS USED IT TO CLEAN UP SPILLS EFFECTIVELY. DOC/NOAA HAS

WITNESSED A SUCCESSFUL DEMONSTRATION OF OSE II REMEDIATING BUNKER C OIL IN SOUTH KOREA. IN EACH CASE ABOVE THEY WERE TESTED OR USED ON FRESH/MEDIUM/HEAVY/WEATHERED TYPES OF OIL.

- Has this agent been used on previous oil spills? AS OF MAY 17TH, 2013, IT HAS BEEN USED TO EFFECTIVELY CLEAN UP 23,600 OIL SPILLS ONFRESH/MEDIUM/HEAVY/WEATHERED TYPES OF OIL.
- What were the characteristics of the oil and the spill environment in each case? FRESH/MEDIUM/HEAVY/WEATHERED TYPES OF OIL ON ROCKY SHORELINE, SANDY BEACH, MARSH, ESTUARINE, RIVERINE, FRESH AND SALT WATER, OPEN OCEAN.
- Are degradation results (based on oil chemistry and microbial tests) available for review? YES, THEY ARE ALL AVAILABLE – A NUMBER OF KEY TESTS ARE CITED IN THE ATTACHED COMPILATION OF DOCUMENTATION OF TEST AND USE RESULTS OF OSE II WITH THE RRT.
- Is a reference available? SEE DOI TEST CITED IN ATTACHED COMPILATION OF DOCUMENTATION OF TEST AND USE RESULTS OF OSE II WITH THE RRT

Supply:

- source of supply OSEI CORPORATION
- amount available – ENOUGH TO CLEAN UP A MILLION GALLONS INITIALLY, WITH COSTED RESUPPLY EVERY 5 DAYS.
- ETA to site – ESTIMATED 2-8 HOURS DEPENDING ON LOCATION OF SITE IN ALASKA

Application:

- estimated amount of agent(s) needed ONE GALLON OF OSE II FOR EVERY 50 GALONS OF OIL OR HYDROCARBON-BASED MATERIAL
- who will apply the agent (vendor personnel, response contractor personnel, or other contractor) VENDOR PERSONNEL OR ANY RESPONSE CONTRACTOR CAPABLE OF APPLYING DISPERSANTS
- method to be used in applying agent ANY TYPE OF SPRAY APPARATUS CAN BE USED, INCLUDING AERIAL.
- impacts of proposed application method – 1) immediate reduction of the toxicity to the environment; 2) causes oil to float protecting the water column and seabed; 3) within 30 minutes, reduces adhesion properties so if oil reaches manmade structures or intertidal zone, or birds and other marine life or wildlife, it will not adhere; 4) diminishes fire hazard in a matter of minutes; 5) permanent removal of the oil/hydrocarbon-based material within a matter of a few days to a few weeks.
- time to prepare agent for application – ready to apply on delivery
- has application equipment been calibrated for this particular application – yes, with most eductor systems. However, if it needs to be changed it is simply a matter of changing the spray dial to 2%.
- planned rate of application – enough to address 500 thousand to a million gallons of oil per day.
- how long will application take – this depends on the amount of oil spilled. For example, if it is 10,000 gallons, it's less than 30 minutes – if it is a million gallons, it would take several hours
- will product have to be reapplied - NO
  - how frequently (not applicable)

**Bioremediation Work plan**

- Has a bioremediation Work plan been prepared? YES – SEE ATTACHED
- Has the plan been reviewed? (IT CAN BE REVIEWED QUICKLY)

**Biomonitoring Plan**

- Has a biomonitoring plan been prepared? YES
- Has it been reviewed? ABSOLUTELY – IT WAS WRITTEN BY THE EPA.

**Project Management**

Bioremediation application project manger: (TO BE FILLED IN PER INCIDENT)
contact number:
address:

This bioremediation application has been approved:

| | | |
|---|---|---|
| Federal On-Scene Coordinator | State On-Scene Coordinator | Environmental Protection Agency |
| Department of Commerce | Department of Interior | |

ATTACHMENTS:

Compilation of Documentation and Use of OSE II with RRT, pages 49-55
Bioremediation Work Plan, pages 56-63
Bioremediation Monitoring Plan, pages 64-79
18 Toxicity Tests can be found at link:
http://osei.us/wp-content/uploads/18-Toxicity-test-with-4-2012-Log0.pdf

**EXHIBIT A**

# COMPILATION OF DOCUMENTATION AND USE OF OSE II WITH RRT

US federal government and RRT groups that have associated with the OSEI Corporations product Oil Spill eater II.

OSE II is on the US EPA National contingency Plan for Oil Spills List.

Link http://www.epagov/oem/content/ncp/products/oseater.htm

This was the **5th time OSE II** had been successfully tested with this same method EPA method to get bioremediation products listed on the EPA NCP list.

EPA contractor utilizing OSE II on US navigable waters, on the Osage Indian Reservation in Oklahoma

**The US EPA also spent millions testing OSE II through NETAC for bioremediation protocol development**. OSE II's successful efficacy tests on Tier II which were peer reviewed by 31 scientist established the fact that OSE II should be tested in Tier II open water mesocosm tests. Tier III initial efficacy tests showed OSE II working well, Tier III also included toxicity testing on two separate marine species, which showed OSE II was practically non toxic. Link http://osei.us/technical-library-documents efficacy tests pages 25-28 and toxicity tests pages 99-101

**EPA/NETAC testing performed by the University of Western Florida** under contract from the US EPA Hap Prichard Gulf Breeze Florida 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 50 was above 5000 showing OSE II is virtually non toxic which is a good indicator of how OSE II would work in the field. This test also tested for degradation of the oil and the tests showed OSE II was impressive at remediating the oil. The link to their test information is http://www.nbiap.vt.edu/brarg/brasym95/kavanaugh95.htm

**Toxicity testing** from the above open water mesocosim effluent as well as toxicity testing the US EPA, Environment Canada, as well as toxicity tests for the South Korean government approval, and others proving OSE II is non toxic to salt water and fresh water species is at the following link http://osei.us/wp-content/uploads/18-Toxicity-test-with-4-2012-Log0.pdf

**Video proof OSE II is not toxic to fresh water marine species or plants**, OSE II being applied directly to the waters surface with Koi fish swimming through the OSE II being applied to oil on the waters surface. This body of water with exotic p[lants and fish has had OSE II applied to it for over 2 years with no adverse effects to the fish or the plants see video at link http://osei.us/archives/1150

**Video proof OSE II is non toxic to salt water marine species, this video also shows OSE II is non toxic to responders, and once OSE II has been applied to oil the oil will not adversely effect humans or adhere to humans as well. The end of the video shows how effective OSE II is at decontaminating equipment made of hydrocarbons and you can see how well OSE II cleans up shorelines as well, See link http://osei.us/archives/1135 see the frames on the video at time 9 minutes and 12 seconds, which shows a small fish swimming under the oil that has OSE II applied with no adverse effects!**

**EPA RRT VII tested OSE II** on heavy waste oil in nine aquariums with pictures of the successful testing of OSE II in triplicate on heavy waste oil with water from a lake, Spring Lake, and from the Missouri River. The NRT/RRTIV guidance documents had stated bioremediation would not remediate heavy oils, the EPA/RRT VII has proven this is not the case.

**EPA/RRT VII See Link**:
http://www.osei.us/pdf%20files/RRT%20plus%20testing.pdf

**EPA Al Venosa literature review of Bioremediation/OSE II** see link
http://www.osei.us/pdf%20files/EPA%20peer%20review%20of%20OSE%20II.pdf

**EPA RRT VI** phone conversation with OSEI CEO Steven Pedigo, Jim Staves, Ragan Broyles, and Steve Mason, Jim Staves stated the EPA could not find a scientific reason why not to use OSE II April 16, 2012!

**EPA RRT IV** personnel viewed a successful demonstration of OSE II on sandy beach and marsh grass contaminated with BP Macondo well oil with Corexit attached. http://osei.us/archives/819

**Oil Spill Eater II demonstrated for members of the Mississippi DEQ and EPA RRT IV members.**

**The US Navy used OSE II on US navigable waters spills in San Diego Bay** on hundreds of spills, with whales and dolphins around without any adverse effects to any marine species while reducing their clean up cost over 87%. The US EPA Debra Dietrich and Nich Nichols met with the Navy officials with the OSEI Corporation in San Diego where EPA officials learned about the 100's of clean ups performed by the US Navy for 3 and ½ years.

Opening link to OSEI home page http://osei.us/

**US Coast Guard Grotten, Conneticut** sent a letter during the BP spill requesting the FOSC to take action with OSE II. The US Coast Guard has purchased and utilized OSE II since 1990 themselves.

**Coast Guard link**
http://www.osei.us/pdf%20files/Coast%20Guard%20BP%20spill%20approval%201.pdf

**Coast Guard Commandent Paul Yost Class mate of Coast Guard (Ret) Admiral Lively of the OSEI Corporation requested the responsible party of the Valdez spill to test OSE II. Exxon tested OSE II in the winter of 1990 in Florham Park New Jersey along with at least 10 other products Exxon thought were the best**

**products in the world. Dr. Brown of the University of Alaska witnessed the test and relayed to us OSE II was 92% more effective than the next best product which included the toxic Inipol product Exxon had purchased the rights for. Exxon understood in the winter of 1990 what product would be the most effective product to clean up the Valdez spill, and because they were not going to make money on it they did not use it.**

**The US Department of Interior** performed a test comparing OSE II to dispersants (Corexit 9527A and 9500A), and mechanical clean up. OSE II cleaned up 67% of the oil while the dispersants were not successful at being effective ( sinking oil into the water column) and the mechanical clean up was able to clean up its normal 2 to 8%. OSE II was proven by DOI to be the superior clean up method. **This RRT trustee's test proves what is the most effective clean up response that meets the Clean water act requirements of permanently removing oil from the environment.** See test summary at link http://osei.us/brochures click on US Department of Interior study, this will allow you to read the summary of the test as well. http://www.google.com/search?client=safari&rls=en&q=OSEI+summary+of+Department+of+interior+test&ie=UTF-8&oe=UTF-8

**US DOI link**

//www.bsee.gov/uploadedFiles/BSEE/Research_and_Training/Technology_Assessment_and_Research/aa(3).pdf

**US NOAA officials visited a demonstration of OSE II, in Mo Hang Port South Korea**, where the gentleman in the yellow jacket the head of the South Korean Coast Guard explained the great successful testing of OSE II, which led to a successful demonstration on the shoreline with South Korean government officials and the approval of OSE II for South Korea as well. These are pictures of NOAA officials wearing NOAA caps at the successful demonstration.

The conclusion of the successful test showed OSE II remediating the Bunker C oil to CO 2 and water, and showed there were small crabs that were living in the water for the duration of the test unharmed. See link http://osei.us/photoalbums/south-korea-hebie-spirit-2 scroll to the bottom of this picture set to see NOAA officials.

**NOAA official Charlie Henry letter.** See link
http://www.osei.us/pdf%20files/NOAA%20Charlie%20Henry%20final%201%2025%202011%20.pdf

**US Department of Energy use of OSE II at their Sunoco Terminal**

**Oil Spill Eater II was used to successfully finish the Clean up of the storage tank mixer failing that produced this spill. The gravel around the storage tank did not have to be removed since OSE remediated the oil from site to CO2 and water.**

**SEE Department of Energy link at http://osei.us/photoalbums/department-of-energy-use-of-ose-ii-2**

**States that have requested the use of OSE II.**

**Valdez Spill**

**Alaska spring of 1990 Alex Viteri of Alaska department of environment quality, requested the EPA and Exxon to do a small demonstration test with OSE II on the Valdez spill, EPA never responded.**

**BP spill**

**State Of Louisiana**

- A letter from the Office of A.G. Crowe requesting the use of OSE II

Louisiana Department of Environmental quality Sanford Phillips requested to demonstrate OSE II at least two times, Sam Coleman EPA RRT VI stated no and Sanford Phillips stated after the last request for the demonstration of OSE II for Sam Coleman to put his response in writing and Sam Coleman refused.

State of Mississippi

- A letter from Senator Gollot of Mississippi requesting the use of OSE II.

State of Alabama

- A letter from Senator Hank Erwin to RRT4 Alabama State Senator support for OSE II

This letter was sent to Unified command after ADAM viewed a demonstration of OSE II and tried OSE II themselves on tar balls from the BP Macondo spill see link http://osei.us/archives/858

**City of Destin after seeing a demonstration of OSE II**

http://osei.us/archives/1005 **The minutes of the special session to view the OSE II demonstration, as well as the minutes from 8/2/2010 where the city council unanimously voted to request to unified command the use of OSE II are under the video.**

- A certified letter from the Office of A.G. Crowe to Barack Obama at The White House demanding the use of OSE II

**Governor Jindal of Louisiana** attempted to have OSE II demonstrated on the BP Macondo spill on May 6, 2010, and RRT VI EPA stopped the Governor from utilizing OSE II to protect his states natural resources.

The preponderance of the evidence, demonstrations, videos, tests, and clean ups on US Navigable waters, as well as all the members of the RRT's that have tested and or utilized OSE II, has proven OSE II is the safer for responders, non toxic to marine species as well as plants, and permanently removes oil from the environment shows OSE II is the means to protect natural resources and return spills sites to pre spill conditions while protecting the environment.

# RRT IV BIOREMEDIATION WORK PLAN UTILIZING BIOREMEDIATION SUB-CATEGORY TYPE ENZYME ADDITIVE – OSE II (applicable for all RRT's)

**Work plans are important to ensure the safe, coordinated, and well documented implementation of bioremediation. Work plans are comprised of systematic procedures and guidelines that clarify and resolve issues such as worker and public safety, documentation requirements, response personnel roles and responsibilities, treatment technique agent application protocols, and application control and oversight considerations. Complete Work plans must include spill and site specific considerations. It is essential in a response that every incident or event be managed according to a plan and bioremediation is no exception. The Work plan shall provide:**

**- A clear statement of objectives and actions.**
**Category EA/ OIL SPILL Eater II's objective in every spill scenario is to address as close to 100% of the spill as possible remediating the spilled hydrocarbon based pollutant to CO2 and water permanently removing the spill from the environment as per the Clean Water Act requirement.**

**- A basis for-measuring work effectiveness and cost effectiveness.**
**Category EA/OSE II requires $2.00 of OSE II for each gallon spilled that Oil Spill Eater II is applied to as of 5/15/2013. The work effectiveness will be readily observable since areas where OSE II has been applied correctly, the oil will be breaking up and separating from the shoreline marsh grass, etc, or in the case of open water spills the oil droplets become so small they become difficult to visually see. An area where the oil would not be breaking up would stand out and could be easily addressed by spraying OSE II to the oil that has not been addressed.**

**- A basis for measuring work progress and for providing accountability.**
**The visual observations of the oil breaking down and lifting from shorelines or disappearing into small droplets in the open waters could be readily viewed. If the oil is not breaking down, then redirecting crews to address these areas with OSE II is easily accomplished.**

**Plans should be prepared for specific time periods or operational periods. These periods can be of various segments of time. Decisions on the length of the operational period or time segments may be affected by the length of time available/needed to achieve objectives, the availability of resources, environmental considerations, and safety considerations.**

**Essential parts of any Work plan:**

**1. Statement of objectives - Statement of what is expected to be achieved. Objectives must be measurable.**

**Category EA/ OIL SPILL Eater II's objective in every spill scenario is to address as close to 100% of the spill as possible remediating the spilled hydrocarbon based pollutant to $CO_2$ and water permanently removing the spill from the environment as per the Clean Water Act, this can be readily viewed or GC/MS can be carried out if needed.**

**2. Organization - Describes what organization will be in place. This will describe in detail the specific roles and responsibilities of the participants in a bioremediation application technique. This will also describe the interaction of one entity to another.**

**The OSEI Corporation will provide a project supervisor, and one consultant for each area polluted by a spill, areas being shoreline, open areas, marshes, estuaries, and special areas. These consultants will oversee each section of the spill (such as the ongoing source of the well blow out for the BP Deepwater Horizon spill). There will be a consultant (deployment manager) that will also make sure, the logistics of staging and supplying application vessels water based or land based are supplied with OSE II in a timely manner. For larger spills this could require two consultants, and for larger spills over 10,000,000 gallons the number of consultants supplied by the OSEI corporation would be roughly one per 10,000,000 gallons of oil spilled.**

**3. Tactics and assignments - Describes tactics and control operations and what resources will be assigned. If the application is a large one, resource assignments may be done by groups.**

**There will be water based vessels for open water spills as well as aerial application, for shorelines land based, and shallow water craft with induction systems will be utilized, marsh and estuaries, shallow water craft with minimal pressure spray application will be utilized. The leading edge of a spill nearest to the shoreline will be address first since it is closest to land fall to prevent the spill from impacting the shoreline.**

**4. Supporting material - Examples include a map or sketch of the area(s) to be treated, communications, traffic plan, weather data, special precautions, and safety information.**

**Satellite mapping with average depth of oil analysis will be carried out as well to determine the amount of oil in any given acre, open water shoreline, or marshes, or tidal flats. Communications covering the most sensitive areas to be protected first from application of category EA/OSE II will be determined and where to place floating curtain booms since the oil will be caused to float allowing booms to actually protect inlets, bays, ports, and harbors, as well as sensate marshes and estuaries.**

**All supervisory personnel must be familiar with the plan and any changes which develop throughout the life of the project. This can be accomplished through briefings and by distributing copies of the written plan. As well as through cell phone and satellite phone communications.**

**The Work plan must include an avenue to provide for ongoing evaluation of the plan's effectiveness.**

**Supervisors should regularly assess work progress against control operations called for in the plan. If deficiencies are found, improved direction or additional staffing may be required, tactical operations may need to be modified, and/or changes may need to be reflected in planning for the next segment of time. Tactical operations could change from wind or a change in current from an incoming storm, otherwise the plan will be consistent with addressing the shoreline and most sensitive areas first.**

**Demobilization activities, although often overlooked, are an integral part of the Work plan. As the project begins to wind down, everyone will be anxious to leave the scene and return home. Demobilization planning helps to assure a controlled, safe, efficient, and cost effective demobilization process. Demobilization will follow from last vessels and personnel to first vessels and personnel until the site is closed.**

**Organization**

**The response structure or organizational framework identifies the participants in a response, their general areas of responsibility, and the lines of authority among them. A chart illustrating the participants in a bioremediation response activity in Region 4 and their inter-relationships would be very helpful in summarizing this information. In developing this section, the following questions should be addressed:**

- **Who will manage the overall bioremediation activity?**

  **With Category EA/OSE II you would need an overall response manager, and open water official, for large spills this would be by areas, and may require more than one official, shoreline, official, ports, and harbor official, marsh and or intake official, official for man made structures, and the OSEI Corporation would present a counter personnel for the same areas.**

**- Who will be the likely participants (e.g. federal and state agencies) in the activity for the Region? What are the general roles?**

**- Who will be the likely participants, if any, from outside the Region? What are the general roles?**

**OSEI Corporation counter part personnel to help direct application areas and actions in concert with federal, state and local officials.**

- **Who will manage the monitoring portions of the activity?**
  **The official for each of the affected areas will also be able to monitor through observation the oil that has been addressed and is breaking down.**

**- Who will develop an appropriate Work plan for the bioremediation**

**activity?**

**The OSEI Corporation will present a plan for each area after satellite information has been obtained; however since category EA/OSE II is safe and has no down sides or trade offs, the immediate application of 1 gallon of OSE II mixed with 50 gallons of non polluted water from near the spill source and then the 1 gallon of mixed OSE II and water application to each spilled gallon can start while the plan is being developed for the particular case/area. The plan will include materials needed, OSE II and water needed, the entire scope of equipment required, which is minimal and readily available, support vessels and craft, or based on availability, the areas to be addressed first to last, and the step by step process for each area, the maintenance if any needed for each area, and testing requested by officials, therefore extraction of samples and the ice chest, sampling jars, labeling, and chain of custody forms and courier for samples to be taken to a designated lab. Sample plan attached.**

- **Who will perform specific treatment method or agent(s) application(s)?**

  **Certified contractors that can already apply dispersants, except shoreline, sensitive areas, and marsh application will be added at the direction of the OSEI Corporation in concert with an RRT official or designate. These vessels and personnel are already in place for dispersants and the transition to utilizing OSE II is easily carried out.**

- **Who will perform monitoring?**

  **The federal, state, local official or designate to work with the OSEI consultant established for each area of the spill. The same officials overseeing the application of OSE II will be able to carry out a duel role, since you can easily observe the effects of applying OSE II to open water, shorelines, marshes/estuaries, and man made structures.**

- **Who will perform public outreach?**

  **The OSEI Corporation will present information every 8 hours of the response, the reactions of the oil/hazardous substance to the application of OSE II and the areas protected, and estimated time to finish addressing an area.**

**Describe in detail the specific roles and responsibilities of the likely participants (RRT, federal and state agencies, international governments/agencies, non-governmental organizations, responsible parties, etc.) in a bioremediation activity in Region 4. The information in this section should coincide with the information presented above on the regional response structure.**

**The role of the designate to work with each OSEI consultant will be to oversee the application of OSE II in each segmented spill area. If there is a specific area of concern in a given area, special attention will be given to address it, so the safest most effective outcome of removing the oil from the environment can be obtained. The designate will be able to oversee application and to revisit**

**application areas to monitor the effectiveness of the application and the oil response to the application. If a given area or spot is not reacting accordingly then a further application will need to be performed in order to make sure 1 gallon of the OSE II and water mixture is making contact with each gallon of oil spilled. OSHA has reviewed the contents of OSE II and has stated there are no concerns with exposure for humans, so responders and officials will be safe without the need for hot chemical suits and respirators. Respirators may be needed initially to protect responders from the oil itself, and the need for this precaution can be gauged by the smell of the oil. Once the smell of the oil is lessened after the application of OSE II has occurred, there is no further need for protecitive respirators.**

**Tactics and assignments**

**Tactical direction includes determining the tactics and operations necessary for the selected strategy and determining and assigning the appropriate resources.**

**Once the spill volume is determined, and the areas it covers, the decision to use aerial spray will be determined. The need for resupply vessels for fuel, food, water and OSE II, etc, the number of aerial spray craft, the number of vessels equipped with spray apparatus similar to dispersant spray apparatus, the number of shallow water vessels and land based application vessels will be determined. The number of vessels will be determined so as to give the spill complete coverage without producing a traffic problem in a given area. A staging area for supplies and OSE II will be established. For large spills this may be numerous areas. An OSEI consultant will be assigned to each area, with an official designate to work with them for application and monitoring, and test extractions if required, and area maintenance, if needed. They will report back every 4 hours, and adjustments can be made by the OSEI Corporation consultant, with agreement from the official designate, or the request for a change in procedure can be requested to the OSEI Corporation spill overseer, and then this can be agreed upon with the official designate. Based on the type of oil, and if the spill event is singular and not ongoing once the oil has had OSE II applied to it with a one to one application of OSE II and water mixture, the oil will start to break up in minutes in the Gulf, and reduce to small particles in open water becoming difficult to see. The oil with OSE II applied in the environment, where OSE II's ability is exponentially more effective than in closed laboratory tests, should have the oil toxicity reducing quickly, and the adhesion properties eliminated in 20 to 30 minutes. This may take a bit longer if it is Bunker C or a very heavy type oil. (In the case of a very heavy oil, 30 minutes after applying OSE II a second application may be needed to force the further breakdown of oil faster, if needed to protect sensitive areas). The importance of adhesion properties reduction is to prevent migratory wildlife from becoming coated in oil. The fact the oil is caused to float prevents the oil from impacting marine species, the water column, the seabed, and its sediments or flora. All during these steps the oil is being converted to CO2 and water, exactly following mother nature's own process - just speeding it up.**

**The plan will be to protect the most sensitive areas, first by applying OSE II to the oil closest to the most sensitive area, along with booming and absorbent barrier strategies, to cut off oil all together from reaching certain areas; then, from an order of first to last of areas that need to be addressed and the vessels, and equipment needed to fulfill a complete removal of the oil from the environment.**

**Resource assignments should be made for each specific work task. Such assignments should consist of the kind, types and numbers of resources available and needed to achieve the desired outcomes. The resources for the actual application will be in the various areas response plan developed by the OSEI Corporation once the scope and type of spill has been determined. Personnel and logistical support factors must be considered in determining tactical operations. Lack of logistical support can mean the difference between success and failure in achieving objectives. There will need to be staging areas for supply, and the personnel to handle the transfer of supply, as well as vessels and their intended use and area. The development of this part of the plan will be derived from the scope and type of oil spilled. The plan can be developed very quickly by the OSEI Corporation based on the facts surrounding the specific spill and will include required materials and equipment.**

**Supporting Material**

**Public Safety/Information - Public safety is paramount in any bioremediation project. The following are some suggested actions that should be taken during a spill response to ensure public awareness and protection: Provide news releases and updates to newspapers, radio, television stations, and neighboring areas that could potentially be impacted by bioremediation activities. Be prepared to discuss details regarding the chosen treatment technique in simple layterms so the affected public will have an understanding of exactly what to expect and what the expected benefits are.**

**Category EA/Oil Spill eater II does not contain any hazardous chemicals as per the country of New Zealand's review, and per the MSDS sheet. This means OSE II itself is not a concern; however, direct contact and inhalation of the oil is of concern and the non responders should be asked to stay away from spill areas, or at least warned of the potential risk with oil spills. Once the application of OSE II is complete in any given area at temperatures above 40°F the oil should be remediated to $CO_2$ and water within 14 to 30 days. At that point the waters would be safe once again for recreational purposes and there would be no residual oil on the sediment to come ashore later. The fish and seabed species would all be safe to harvest since the oil would have never encroached their living areas, and or they would have been able to escape the oil's toxicity since it would be held on the surface and prevented from entering the water column or seabed.**

**Site/Worker Safety - Worker health and safety is always the foremost concern during any spill response action. Since all oil spill response actions require a health and safety plan and the bioremediation application is merely a facet of the total spill response effort, the existing heath and safety plan should be used for**

**the bioremediation application and augmented with the specific safety hazards associated with the bioremediation treatment method or agent application. A section referred to as biological hazards should be included in all health and safety plans associated with oil spill responses where biological agents are used as a response tool. This section should discuss the specific health and safety concerns associated with possible exposure to biological agents and include material safety data sheets (MSDS) for all agents being used.**

**At a minimum, the health and safety plan should address the following aspects of the bioremediation treatment method/monitoring program:**

**1. Minimum health and safety concerns:**

**Any concerns regarding health and safety would be because of the oil, not OSE II. OSE II is completely non-toxic - you can wash your hands with it and handle OSE II with no harmful effects whatsoever.**

**2. Potential hazards during application and monitoring:**

**There are no potential hazards from OSE II itself. There are, however, from the oil, and direct contact with the oil should be avoided.**

**3. Evaluations of those identified hazards:**

**Any hazards related to oil spill cleanup with OSE II are solely from the oil itself and not OSE II. Direct contact with the oil should be avoided.**

**4. Actions described to minimize the potential hazards:**

**Goggles to avoid gaseous oil vapors should be worn to avoid irritation of the eyes.**
**A mask should be worn to prevent inhalation of oil vapors. Gloves should be worn to avoid any direct contact with oil.**
**For those responders only dealing with the supply line of OSE II to the site, etc., no Haz Mat equipment of any kind is required because OSE II is totally non-toxic and safe for responders.**

**5. Response(s) needed if hazard does affect worker(s).**

**Category EA/OSE II, in and of itself, is not a hazardous material and it does not contain any hazardous chemicals. After reviewing OSE II's matrices, OSHA has stated that OSE II does not pose any potential harm to humans.**
**However, when around an oil spill, we suggest gloves in case of contact with the oil, goggles to protect from oil/gas vapors, and a mask to protect from oil vapors. Neither Chemical respirators nor chemical suits are required. Normal hygienic practices should be carried out. Any gloves or clothing, shoes or boots that come into contact with the oil can be safely cleaned off with OSE II. The oil is the hazard of concern, not OSE II.**

**The following documents contain guidance on the preparation of health**

**and safety plans:**

**1. OSHA 1910.120 and EPA 40 CFR 311,**
**2. USEPA, OERR ERT Standard Operating Procedures,**
**3. NIOSH/OSHA/USCG/EPA Occupational Health and Safety Guidelines,**
**4. ACGIH Threshold Limit Values, and this would be needed for the oil not OSE II.**
**5. existing local and area contingency plans.**

**To avoid disturbances to the treated area after treatment, all treated and control sites should be secured by the best achievable means. To avoid possible injury, post warning signs or secure the treated area to differentiate the site from surrounding localities.**

# Bioremediation Monitoring Plan for

# Bioremediation, Sub-category EA Enzyme Additive - OSE II

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

JAN 16 1992

OFFICE OF
SOLID WASTE AND EMERGENCY RESPONSE

MEMORANDUM

SUBJECT: Region 6 Bioremediation Spill Response Plan

FROM: Karen Sahatjian, Chair
Implementation Subgroup, Subcommittee on National Bioremediation Spill Response

TO: Members of the Implementation Subgroup

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Again, I would like to thank those members of the Implementation Subgroup who attended our meeting on Thursday, December 5, and Friday, December 6, 1991, in New Orleans, Louisiana. We have made great strides over the past few months in developing the Region 6 Plan and I sincerely thank everyone for their participation.

Attached for your review is the latest draft of the Plan. I would appreciate your careful attention in reviewing the draft, as I would like to present the Plan on behalf of the Subgroup in final form to the Regional Response Team next month. As we discussed at the New Orleans meeting, I have set up a conference call on January 28 to discuss any comments or concerns you may have regarding the Plan. Please call (202) 269-4246 at 2 p.m. Eastern time to be connected into the conference. The conference call has been scheduled for 2 to 3:30 p.m.

Please feel free to call me at (202) 260-1354 if you have comments or questions. Once again, thank you very much for contributing your time and effort to this important project.

Attachment



Printed on Recycled Paper

# SECTION 6

# MONITORING

Bioremediation is often assumed to enhance the biodegradation of oil or hazardous substances without increasing adverse impacts to human or ecological health. Until there is evidence to confirm this assumption, however, bioremediation effectiveness and safety need to be monitored through a sound program of applied science.

This section presents a general plan that provides Federal On-Scene Coordinators (FOSCs) with the information needed to prepare for, implement, and oversee monitoring activities designed to enable the objective evaluation of bioremediation in the response to a spill. (Please note that these activities should be coordinated with the activities of the Shoreline Cleanup Assessment Team.) The plan is intended primarily for monitoring oil spill bioremediation activities conducted in coastal areas and estuaries. Preparing for monitoring in advance of a spill is stressed in order to reduce the number of decisions that must be made during an actual response, increase the effectiveness of monitoring efforts, and promote the use of standard, accepted protocols.

The plan presented in this section is intended to be implemented primarily through the responsible party's or the FOSC's contractor. The contractor will use this plan as a minimum scope of work to develop a detailed, site-specific workplan (the specific elements of the workplan will be developed through negotiation with the monitoring Project Manager). If an agency of the Regional Response Team (RRT) decides to implement a bioremediation monitoring plan with its own resources, that agency will also need to develop such a workplan.

## 6.1 OBJECTIVES

The principal objectives of the monitoring program and the major elements of each objective are listed below.

Objective 1: Determine the efficacy of the selected bioremediation agent in enhancing the degradation of spilled contaminants.

- Certify the viability of microorganisms and/or nutrient composition of the bioremediation agent in a laboratory setting before or concurrent with the initial application; Done → this has been accomplished repeatedly
- Certify the ability of the bioremediation agent to degrade or enhance the degradation of spilled contaminants in a laboratory setting before or concurrent with the initial application; and Done → this has been accomplished repeatedly
- Determine the extent to which the bioremediation agent has enhanced the rate of contaminant biodegradation as compared to an untreated, contaminated site.

Objective 2: Measure the environmental impact of bioremediation treatment for the duration of the monitoring activity. Dispersant toxicity tests with OSEII for the EPA show the oil toxicity is reduced by 10000 in 24 hours

- Determine the extent to which the bioremediation agent increases or decreases the toxicity of spilled contaminants; OSEII reduces

- Document adverse physical effects attributable to bioremediation agent application and monitoring activities; and in over 16,000 spill cleanups over 22 years there has never been ANY adverse effect with the use of OSEII
- Determine the extent to which the bioremediation agent alters the nutrient dynamics of the treated habitat. OSEII by causing oil to float prevents the depletion of O2 in the water column & keeps the oil & OSEII on the surface.

Objective 3: Ensure the comparability of data collected from all monitoring projects in the Region for use in a Region 6 bioremediation data base. OSEII really does not effect nutrient levels as tests have proven

- Employ standard methods and operating procedures at all monitoring projects; and
- Conduct sampling both at replicate treated and untreated (control) sites for all bioremediation activities, unless replication is specifically ruled out on the basis of informed judgement by the FOSC or the monitoring Project Manager. Sampling from replicate sites is needed to establish variance of means among sites.

## 6.2 USES OF COLLECTED DATA

The primary use of monitoring data will be for response management decision-making by the FOSC. Properly collected, validated, and interpreted data provide critical information to assess the efficacy and environmental impact of bioremediation treatment and related response activities. Such documentation is needed to identify and correct problems in the biological treatment process, to determine whether bioremediation end-points have been reached, to ensure that biotreatment is less environmentally harmful than the spilled pollutant, and to support cost recovery and other legal actions.

Secondarily, the data can also be used for developing regional and national data bases, interfacing with natural resource trustees, preparing interim and final reports, and revising this monitoring plan.

## 6.3 MONITORING PLAN DESIGN

OSEII was successfully tested & monitored through EPA/NETAC Tiers III & IV

The monitoring plan described in this section is designed to be implemented in various levels of response based nominally on spill volume. The rationale for this design is that increasingly more comprehensive monitoring will be necessary and should be undertaken as the volume of a spill increases (assuming that the size of any bioremediation activity also increases), or as the potential for damage to sensitive resources attributable to the spilled oil or bioremediation activity increases, regardless of spill volume. (Weather conditions, the location of a spill, and the particular location of any ensuing bioremediation activity also need to be considered when determining the appropriate monitoring response level.) In addition, the design provides flexibility to tailor monitoring activities to best fit the conditions associated with a particular bioremediation activity.

Because a principal goal of monitoring is to establish whether the addition of bioremediation agents accelerates contaminant degradation without contributing significant adverse environmental impacts, the monitoring plan design provides for the comparison of data from replicate treated and untreated areas throughout the duration of a bioremediation activity. That is, the plan proposes that observations be made and samples collected and analyzed for: (1) uncontaminated, untreated source areas; (2) contaminated, untreated source areas; and (3) contaminated, treated source areas. This approach should be followed for each bioremediation activity and monitoring response level to the extent possible. after 16,000 spill cleanups, and numerous efficacy studies, that by EPA & other governments OSET has proven beyond a shadow of a doubt that OSET accelerates contamination degradation without adverse effects to the environment or responders

Details of each monitoring response level and the criteria for selecting treated and untreated sites are provided below.

### 6.3.1 Monitoring Intensity Levels

Monitoring intensity levels describe the scale of field and laboratory activities that should be performed as part of the monitoring effort of a bioremediation activity. Monitoring intensity levels vary primarily with the size of the monitoring effort, rather than the specific types of activities to be performed. In this context, the "size" of the monitoring effort refers to the number of samples to be taken and the sampling density, as well as resource requirements needed to accommodate increased sampling and analysis activity.

The activities proposed for monitoring intensity levels assume that any bioremediation agent used is both listed on the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule and has undergone controlled laboratory testing that at least demonstrates its ability to degrade oil. Generally, it is assumed that bioremediation agents have not undergone adequate field testing to demonstrate their efficacy and safety.

**Intensity I Monitoring Response -- Spill <240 Barrels**

Historically, oil spills smaller than 240 barrels (or 10,000 gallons) have been the most common. Bioremediation activities following a spill of less than 240 barrels could be undertaken, for example, to clean up habitats, such as sensitive marshes, where mechanical methods would be inaccessible or too disturbing to be practical, or to hasten cleanup of lightly-oiled shorelines outside of public-use areas. Monitoring of a bioremediation activity following an Intensity I spill should, at a minimum, incorporate the following activities:

**Field Activities**

Reconnaissance -- collection of screening and qualitative information through a preliminary survey of the spill area. Information collected will be used to assist in designating treatment and control sites, evaluating logistics of monitoring, and determining resource needs. Reconnaissance activities should include performing visual observation from aircraft or boat; tracking oil distribution and movement; assessing the presence, location, and abundance of spilled material; and evaluating potential logistical problems posed by the physical habitat. Generally, this type of information will be collected by the Shoreline Cleanup Assessment Team as part of the monitoring for the overall response to a spill. Therefore, reconnaissance for bioremediation monitoring should be coordinated

with these other information collection activities to minimize any unnecessary duplication of effort.

Environmental parameters assessment -- collection of information on weather conditions and measurement of field conditions or water quality, where applicable, to assist in selecting treatment and control sites and, later, in evaluating effects of bioremediation agent applications.

Sampling -- collection of water, sediment, and/or shoreline-material samples on which to perform laboratory efficacy and toxicity tests to evaluate effects of bioremediation agent applications, as well as nutrient balance analyses. Samples should be collected in triplicate at each sampling site, water depth (as appropriate), and time. For applications in marshes or shallow waters, surface water and the top two centimeters of sediment should be collected. For applications on beaches or shorelines, only shoreline material (e.g., sand, shell) needs to be collected. For applications on enclosed open water (such as bays), surface and bottom water samples should be collected to the extent practical; at a minimum, where water is less than 20 to 30 feet deep, pretreatment surface water samples and sediments should be collected.

Other visual observation and documentation -- collection of qualitative information on environmental characteristics to help evaluate the effects of bioremediation agent applications and ensure that the spill situation and ensuing response are documented thoroughly and completely. Visual observations and measurements should focus on assessing readily discernable effects of oil and oil plus bioremediation agent on indigenous biota; physical effects associated specifically with agent applications and monitoring activities; presence, location, and abundance of spilled material; appearance changes (such as percent change in color, stickiness, and consistency) in spilled contaminants and bioremediation agent materials; and distribution and movement of spilled contaminants and bioremediation agent materials. Documentation shall be collected and assembled over the period of the response.

**Laboratory Activities**

Efficacy testing -- use standard EPA-approved laboratory protocols or other validated and accepted methods to analyze collected samples to measure relative changes in the: (1) composition and amount of spilled oil to assess the effectiveness of bioremediation agent applications; and (2) nutrient concentration to assess effects of agent applications on ambient concentrations and the adequacy of the application strategy to maintain microbial growth and degradative activity.

Toxicity testing -- use standard EPA-approved laboratory protocols to analyze collected samples to assess and confirm the presence or absence of toxicological effects associated with bioremediation agent applications relative to those associated only with the spilled oil.

**Intensity II Monitoring Response -- Spill 240 to 2,400 Barrels**

As the size of a spill increases, the likelihood of adverse effects that are attributable to the spilled oil also increases. Consequently, it is possible that several distinct habitats or sensitive

resources may be threatened or affected by spilled oil and that bioremediation may be considered for treating more than one of them. The number of unique bioremediation activities and, particularly, the scale of any bioremediation activities that may follow a spill of 240 to 2,400 barrels (or 10,000 to 100,000 gallons) are likely to exceed those that may follow an Intensity I spill. The approach to monitoring should more carefully consider the potential merit of applying bioremediation agents in particular environments or of applying particular agents as part of the overall spill cleanup strategy. A two-phased approach is proposed.

**Phase I Activities**

This phase provides for a pilot or small-scale field test to be conducted with each unique bioremediation agent or for each distinct habitat proposed for treatment (depending on the scale of application planned and its potential effects). For example, a bioremediation activity to treat the entire area of a 500-barrel spill should be preceded by a small-scale field test. Alternatively, a bioremediation activity to treat only a one acre area of a 500-barrel spill that does not encroach on any sensitive resources would probably not require an initial field test.

Specific monitoring activities to be performed include the following, as defined above:

- Reconnaissance, results of which will be used to designate both the location and size of test plots (one-fifteenth the area proposed for full-scale treatment is suggested; however, the area may be larger depending on the overall size of the proposed treatment area);
- Sampling; and
- Efficacy and toxicity laboratory testing, focusing on analysis of trends over the test period.

**Phase II Activities**

Based on results of Phase I field applications and monitoring, bioremediation agents may be applied on a larger scale and to several oiled habitats. The types of monitoring activities conducted under this phase should be the same as those conducted for an Intensity I Monitoring Response. The monitoring regime should be repeated for each distinct habitat that is treated.

**Intensity III Monitoring Response -- Spill >2,400 Barrels**

A spill of this size may require a multiplication of the level of effort outlined for an Intensity II spill (i.e., several small-scale field tests -- one for each habitat considered for treatment or each bioremediation agent considered for use -- and several monitoring teams with appropriate equipment and supplies to collect samples and make observations). If the FOSC recommends and the RRT concurs that equipment, personnel, and financial resources needed to conduct recommended monitoring cannot be obtained, monitoring could be performed on fewer sites as long as these sites are representative of treated habitats and allow for appropriate controls. The same types of field and laboratory activities described for Intensity II monitoring should still be performed.

### 6.3.2 Selection of Treated and Untreated Sites

Treated and untreated (or control) sites should exhibit similar chemical and physical characteristics to support their comparability. Preferably, a number of unique treated and untreated sites should be selected for each significantly different habitat intended for bioremediation treatment. To select treatment and control sites, the following are among the criteria that should be considered: (1) environmental parameters; (2) physical habitat and geological morphology; and (3) oil loading and the probability of further oiling.

Chemical characteristics of the spill environment as well as temperature may influence the effectiveness of bioremediation treatment. For aquatic spills, whether in enclosed open water, coastal areas, or estuaries, try to ensure that the variability in the following environmental parameters between sites is no greater than indicated below:[12]

- Dissolved $O_2$ concentration -- ±2 to 3 ppm (should be ±1 ppm);
- Salinity -- ±3 to 5 ppt (should be ±1 to 3 ppt); and
- Temperature -- ±3 to 5°C (should be ±1 to 3°C).

The physical habitat and geological morphology of the spill area can affect: (1) the extent of contact between contaminants and potential microbial degraders; (2) the potential for contaminant or bioremediation agent migration from or into test areas; (3) the ease and success of agent application and sampling efforts; and (4) the potential for unexplainable variances in observation and sample analysis results. Potential variances between test areas attributable to wave action, tidal flushing, currents, boat traffic, and exposure to wind or other external forces also should be considered and minimized, where possible, in selecting test sites.

Because efficacy analyses focus on evaluating relative changes in the concentration of the constituents of oil between treated and untreated sites, it is important to ensure that: (1) uncontaminated source areas remain uncontaminated for the duration of the monitoring program; and (2) contaminated areas, upon selection, are similarly oiled, and are not re-oiled for the duration of the monitoring program (otherwise, monitoring will need to be re-initiated). Uncontaminated control areas should be carefully selected to minimize the potential of contamination. Booming of control areas may be helpful. The selection of contaminated areas should be restricted to those with uniform oiling (i.e., ±10 to 20% difference). To lessen the probability of further oiling of treatment or control areas, the selection of treatment and control source areas proximate to any of the following should be avoided if possible:

- Inflows of water or runoff;
- Petroleum discharge sources; and
- Marinas and fish camps.

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[12] Suggested maximum variations for these environmental parameters were recommend by Jim Clark of EPA's environmental laboratory in Gulf Breeze, Florida, with concurrence of the Monitoring Workgroup of the Subcommittee on National Bioremediation Spill Response's Implementation Subgroup.

## 6.4 MONITORING PARAMETERS AND COLLECTION FREQUENCY

The environmental characteristics and measurements that should be assessed and the samples that should be taken as part of the field monitoring activities are presented in Exhibit 6-1, along with a schedule for performing these activities. Sampling at each site, water depth (as appropriate), and time should be performed in triplicate. Although the size of samples collected should be based on the requirements of the analytical methods to be used for their analysis, the sizes of 1 liter for water samples and 20 grams (or 20 milliliters) for sediment or shoreline-material samples are recommended minimums. All samples should be collected in methylene chloride-rinsed jars or bottles with teflon-lined caps, as appropriate.

Parameters and methods for performing laboratory analyses of samples collected are presented in Exhibit 6-2. Copies of analytical methods are provided in Appendix F. Other methods are currently being developed by the National Environmental Technology Applications Corporation in coordination with EPA.

## 6.5 DATA QUALITY REQUIREMENTS AND ASSESSMENTS

All data collection activities must be planned and conducted to produce data of known and acceptable quality. To help ensure that these objectives are meet, all contractors performing work as part of the monitoring effort must submit to EPA and the lead agency from the affected state a quality assurance plan. Parameters for defining data quality include precision, accuracy, representativeness, comparability, and completeness.

Representativeness and comparability have been designed into this monitoring plan through provisions for replicate sampling from treated and untreated areas and the use of standard, approved methods for sampling and laboratory analyses.

[DATA QUALITY REQUIREMENTS FOR EACH TYPE OF MEASUREMENT MADE DURING A BIOREMEDIATION ACTIVITY DEFINED BY PRECISION, ACCURACY, AND COMPLETENESS ARE TO BE DEVELOPED BY THE REGION]

## 6.6 SAMPLE CUSTODY PROCEDURES

Accurate identification and proper control of samples is important to help ensure the acceptability and usability of the resulting analytical data. Having standard sample custody procedures is particularly important where the individuals performing sample collection may vary and where individuals collecting samples will not be the ones analyzing the samples. Where the monitoring program is conducted by a contractor, the contractor should designate a sample custodian who will ensure that custody procedures are properly followed.

[SAMPLE CUSTODY PROCEDURES OUTLINING THE METHODS FOR IDENTIFYING AND TRACKING SAMPLES, VERIFYING PROPER LABELING OF SAMPLES, AND ARCHIVING SAMPLES ARE TO BE DEVELOPED BY THE REGION]

## EXHIBIT 6-1

## FIELD MONITORING PARAMETERS

| Parameter | Sample Size[1] | Assessment/Collection Location | Assessment/Collection Frequency[2] |
|---|---|---|---|
| Visual observations (mortality, behavioral effects, appearance changes, oil distribution) | N/A | All test sites | Daily to the extent possible; at least each day that water, sediment, and/or shoreline material sampling is performed |
| Temperature (air, water) | N/A | All test sites | Days 0, 1, 4, 10, and 20 |
| Salinity | N/A | All test sites | Days 0, 1, 4, 10, and 20 |
| Dissolved oxygen | N/A | All test sites | Days 0, 1, 4, 10, and 20 |
| Sea state | N/A | Activity area | Days 0, 1, 4, 10, and 20 |
| Current | N/A | Activity area | Days 0, 1, 4, 10, and 20 |
| Wind velocity | N/A | Activity area | Days 0, 1, 4, 10, and 20 |
| Efficacy (water, sediment, and/or shoreline material) | 1 liter water; 20 grams sediment or shoreline material | All test sites and, as appropriate, all water depths | Days 0, 4, 10, and 20 |
| Toxicity (water, sediment, and/or shoreline material) | 8 liters water; 20 grams sediment or shoreline material | All test sites and, as appropriate, all water depths | Days 0, 1, and 4 |

[1] N/A means "Not Applicable."
[2] Frequency is relative to the time of agent application.

EXHIBIT 6-2

LABORATORY ANALYSIS PARAMETERS

| Parameter | Sample Matrix | Methodology | Recommended Methods |
|---|---|---|---|
| Oil hydrocarbons (C17, pristane, C18, phytane) | Water<br>Sediment/shoreline material | GC + GC/MS<br>GC + GC/MS | ASTM Method D3328<br>ASTM Method D3328 |
| $NH_3$ | Water<br>Sediment/shoreline material | Spectrophotometric<br>Spectrophotometric | |
| $NO_3$ | Water<br>Sediment/shoreline material | Spectrophotometric<br>Spectrophotometric | |
| $NO_2$ | Water<br>Sediment/shoreline material | Spectrophotometric<br>Spectrophotometric | |
| $PO_4$ | Water<br>Sediment/shoreline material | Spectrophotometric<br>Spectrophotometric | |
| Toxicity | Water<br>Sediment/shoreline material | 4-day acute or 7-day chronic<br>4-day acute or 7-day chronic | |

## 6.7 SAMPLING AND ANALYTICAL METHODS

All sampling and laboratory analyses should follow EPA or other approved methods, unless otherwise stipulated or requested by the FOSC.

[RECOMMENDED SAMPLING AND ANALYTICAL METHODS WILL BE PROPOSED AT A LATER DATE]

## 6.8 RESPONSE ORGANIZATION AND RESOURCE REQUIREMENTS

For federalized spills, the decision to use bioremediation is made in accordance with the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) and the Region 6 Regional Contingency Plan (RCP). Once the decision and approval to bioremediate are final, the monitoring plan should be implemented. The RRT member agency that proposes the use of bioremediation on a particular spill will provide a Project Manager to implement the monitoring plan, subject to the approval of the FOSC. Personnel comprising monitoring teams and equipment resources to conduct monitoring will be provided by the RRT member agencies and contractors, as appropriate.

Specific responsibilities of the monitoring Project Manager include the following:

- Obtain approval from the FOSC for the monitoring plan;
- Assemble monitoring teams to perform observations and sampling, as appropriate, to successfully meet monitoring objectives;
- Coordinate all activities with the FOSC to ensure that monitoring does not interfere with other on-going or planned response operations;
- Name a sample custodian to coordinate all sample transfers and chain of custody;
- Ensure that monitoring teams have representation from each RRT member agency that wishes to participate;
- Provide a continuous communication link with the FOSC to ensure timely transfer of monitoring data and results that are relevant to response operations;
- Act as a liaison with natural resource trustees;
- Ensure that the quality of environmental data is known, documented, and sufficient to meet the requirements of the data users and decision makers; and
- Ensure the preparation and submission to the FOSC of all required reports on the monitoring effort.

Monitoring team members will be responsible for implementing this monitoring plan and any other bioremediation activity-specific procedures as directed by the Project Manager, ensuring

the quality of samples and data collected, and participating in the preparation and review of all required reports on the monitoring effort.

### 6.8.1 Personnel Requirements

The suggested minimum number of qualified personnel (in addition to the Project Manager) for carrying out the field activities associated with each monitoring response intensity level are listed below. Specific qualification requirements shall be provided by the RRT. In general, personnel responsible for making visual observations and measurements need to be trained or experienced in conducting physical observations in the field. Personnel responsible for collecting samples need to be properly trained and experienced in the collection of water, sediment, and shoreline material, as appropriate. Wherever possible, the same crews should conduct observations and sampling throughout the monitoring effort.

**Intensity I Monitoring Response -- Spill <240 Barrels**

- 2 people to conduct visual observations and appropriate documentation.
- 2 to 3 people to conduct land-based sample collection, as appropriate.
- 4 people to conduct water-based sample collection (2 boat operators and 2 sample collectors), as appropriate.

**Intensity II and III Monitoring Responses -- Spills ≥240 Barrels**

Personnel requirements for these levels of response will depend on the number and scale of each unique bioremediation activity undertaken simultaneously following a spill. The personnel requirements proposed for an Intensity I response should be used as a baseline and scaled-up as appropriate.

### 6.8.2 Minimum Equipment Requirements

RRT member agencies, other state agencies, and/or contractors that may oversee or participate in the monitoring for a bioremediation activity should be prepared to provide equipment resources necessary to conduct monitoring. The following equipment and supplies at a minimum should be assembled and be made ready for transport to the field to support a monitoring effort:

- Anemometers;
- Binoculars;
- Buckets (five-gallon size);
- Calculator;
- Camera (35 mm SLR) with film and appropriate filters;
- Cassette recorder (portable) with appropriate accessories;
- Cellular telephones and/or portable radios;
- Chain-of-custody forms;
- Chain-of-custody seals;

- Clipboard;
- Compass;
- Current meter;
- Field notebook;
- First-aid kit;
- Flashlight with batteries and spare bulb;
- Ice chest and ready access to ice;
- Kemmerer sampler or Van Dorn bottle, preferably stainless steel;
- Mercury thermometer (-5° to 45°C);
- Paper towels;
- Pens, pencils, and markers;
- Personnel safety equipment;
- Plastic sheeting and rubber bands;
- Polyvinyl chloride pipe, large diameter;
- Portable CTD or DO meter (or Winkler kit), pH meter, and conductivity meter (or refractometer));
- Resealable plastic bags;
- Sample containers (cubitainers, VOA vials, methylene chloride-rinsed one-liter jars, and methylene chloride-rinsed five-liter jars with silicone rubber "O" rings, silicone drain tubes, and teflon-lined lids);
- Sample preservatives;
- Shipping labels;
- Tape;
- Towels or rags;
- Video camera with tape, batteries, etc.;
- Vessels complete with communication and navigation equipment as appropriate for offshore motoring; and
- Watch.

## 6.9 DATA VALIDATION

All data will be subject to a thorough check by the FOSC and the monitoring Project Manager, or their designated representative, for errors in transcription, calculation, or computer input. In addition, the Project Manager will review all incident logs, sample logs, and data forms to ensure that requirements for documentation and data quality assessment have been met.

## 6.10 PERFORMANCE AND SYSTEM AUDITS

To help ensure that work being performed -- whether by contractor, EPA, or state personnel -- is progressing in accordance with the monitoring plan and any specified objectives or procedures, the FOSC, through the designated monitoring Project Manager, maintains the right to conduct performance or system audits of field and laboratory data collection activities. The category of audits are described below:

Management System Reviews -- evaluate the Quality Assurance Program of an organization, such as a firm contracted to conduct a monitoring project or laboratory sample analyses. The purpose of this review is to verify whether the quality assurance management procedures stated by contractor are in place, prior to a contract award.

Data Quality Audits -- evaluate a data set, or all data sets, of a particular project, by comparing the data set against specified data quality requirements for that data set.

Technical System Audits -- evaluate the actual environmental measurement data-collection systems and their associated quality control systems. These audits involve on-site auditing of field sampling activities, field measurement activities, and laboratory analytical procedures.

Performance Audits -- evaluate analytical methods and procedures of a laboratory. These audits are conducted by submitting performance evaluation samples to a laboratory for analysis. The samples contain specific pollutants in known matrices whose concentration and identity are unknown to the testing laboratory (the identity and concentration of pollutants is known to the submitter, however).

[PROVISIONS FOR PERFORMANCE AUDITS AND INTERNAL SYSTEM REVIEWS TO BE CONDUCTED BY THE MONITORING PROJECT MANAGER OR OTHER QUALITY ASSURANCE PERSONNEL ARE TO BE DEVELOPED BY THE REGION]

## 6.11 DOCUMENTATION AND REPORTING

During the course of a bioremediation activity and accompanying monitoring effort, the following reports should be prepared and submitted to the FOSC:

Activity reports -- provide descriptions of the bioremediation activity area, weather, unique observations, and activities undertaken, as well as the names, affiliations and signatures of persons on site. Activity reports should be prepared whenever activities on a site are undertaken.

Analytical reports -- provide laboratory analysis results of environmental and control samples. Analytical reports should be prepared and submitted by the analytical lab within 10 days after receipt of environmental samples for analysis.

After action report -- provides a description of the overall bioremediation activity and accompanying monitoring effort, including results of both field and laboratory activities. An interim draft should be submitted within 30 days after the end of the monitoring effort. A final draft (incorporating comments from the FOSC, RRT members, and other entities involved in the monitoring effort as well as photos) should be submitted within 60 days after submission of the interim draft. As to the discretion of the FOSC and the monitoring Project Manager, however, the time for submitting the final draft may vary depending on whether comments on the interim draft are received in a timely manner.

In addition, at the time the final after action report is submitted, all field notes, including those of contractors, should be submitted to the FOSC.

To facilitate information transfer and the development of a data base on bioremediation use and bioremediation agents, the Bioremediation Use Follow-up Form in Appendix G should be completed at the end of a bioremediation activity.

## 6.12 REVISING PLANS AND PROCEDURES

The monitoring plan and suggested procedures outlined in this section should be implemented and modified, as necessary, based on the cumulative experience and knowledge gained from conducting bioremediation field activities and associated laboratory activities. Recommendations for revisions should be submitted to the Region 6 RRT for approval. Upon approval by the RRT, revisions should be incorporated into the Region 6 RCP and other local contingency plans, as appropriate.

For Open water monitoring and testing the dispersant monitoring protocol can be used for category EA/OSE II as well.

# APPENDIX C

# EVALUATING BIODEGRADATION POTENTIAL OF VARIOUS OILS

# APPENDIX D

# BIOREMEDIATION AGENTS AND AGENT SELECTION

This section describes the various types of bioremediation agents, a procedure for evaluating them, and guidelines for selecting the appropriate agent for use in a particular spill situation.

## Background

Section 311 of the Clean Water Act requires that the US Environmental Protection Agency (EPA) prepare a schedule of dispersants and other chemicals that may be used in preparing for and responding to discharges of oil and releases of hazardous substances, as provided for in the National Oil and Hazardous Substances Pollution Contingency Plan (NCP), 40 CFR Part 300. This schedule is known as the NCP Product Schedule. The Schedule lists agents that may be authorized for use on oil discharges in accordance with the procedures set forth in Section 300.910 of the NCP. (Authorization of use requires that the Federal On-Scene Coordinator (OSC) considering the use of a dispersant or other agent, such as a biodegradation enhancing agent, seek the concurrence of the Regional Response Team prior to the agent's application.) Any agent considered for application to an oil spill should be listed on the NCP Product Schedule.

The NCP Product Schedule currently divides chemical and biological agents into five categories:

1. dispersants,
2. surface collecting agents,
3. biological additives,
4. burning agents, and
5. miscellaneous oil spill control agents.

Most bioremediation agents, including those that are solely nutrients, are listed as biological additives, as the designed purpose of these agents is to enhance the rate of oil biodegradation by increasing microbial activity. There are also bioremediation agents listed as dispersants; these agents are water-based products that claim to enhance the rate of oil biodegradation by emulsifying spilled oil thereby making it more "bio-available." Additionally, other products that do not fit a current regulatory definition because of their unique nature may be listed as miscellaneous agents. Use of any of these agents should be consistent with the Regional Response Team's general guidelines for their application and use.

## Types of Agents

The number and type of agents which claim to enhance the rate of

biodegradation has broadened to fill the current perceived market. Although there are no current regulatory definitions for every type of bioremediation agent, the following are broad definitions for those currently available:

**Microbial Agents** -- concentrated cultures of oil-degrading microorganisms grown on a hydrocarbon-containing medium that have been air- or freeze-dried onto a carrier (e.g., bran, cornstarch, oatmeal). In some cases, the microorganisms may be grown-up in bioreactors at the spill site. All commercially available agents use naturally-occurring microorganisms. Some agents may also contain nutrients to assure the activity of their microbial cultures. This type of agent is intended to provide a massive inoculum of oil degrading microbes to the affected area thereby increasing the oil-degrading population to a level where the spilled oil will be used as a primary source of food for energy. Microbial agents are designed to enhance the biodegradation of oil at any, location and would be most useful in areas where the population of indigenous oil degraders is small.

**Nutrients** -- agents containing nitrogen and/or phosphorous as the primary means to enhance the rate of growth of indigenous oil-degrading microorganisms. This type of agent is intended to increase the oil-degrading biomass already present in an affected area to a level where the oil will be used as a primary source of food or energy. Because the natural environment may not have sufficient nutrients to encourage bacterial metabolism and growth, extra nutrients may be required. The purpose of this type of agent, therefore, is to provide the nutrients necessary to maintain or increase microbial activity and the natural biodegradation rate of spilled oil. This type of product has been used in Prince William Sound, Alaska and Pall's Island, New Jersey to reduce the amount of oil on contaminated beaches. [For information on uses in Alaska, refer to Pritchard and Costa's article entitled 'EPA's Alaska Oil Spill Bioremediation Project' in *Environmental Science & Technology* (Vol. 25(3), 1991), and the article by Chianelli *et al.,* entitled "Bioremediation Technology Development and Application to the Alaskan Spill" *in Proceedings: 1991 Oil Spill Conference.*]

**Enzymatic** - bio-catalysts designed to enhance the emulsification and/or dispersion of oil and make it more available to microorganisms as a source of food or energy. These agents are generally liquid concentrates, which may be mixed with surfactants and nutrients that are manufactured through fermentation. This type of agent is intended to enhance biodegradation by indigenous microorganisms.

**Other Agent** -- include agents that do not fall under the above definitions, such as application mechanism agents that are designed to have an affinity for oil and bring together the elements needed for enhanced oil degradation. Examples of application mechanism agents include time release capsules, liposomes, timed-release fertilizers (e.g., Custom blend), and agents that make oil more hydrophilic.

See pages 6-12 of this document for a more complete evaluation of bioremediation products and their applicable abilities - Types of Bioremediation Products and their mode of action.

### Agent Evaluation Procedure

In considering bioremediation agents listed on the NCP Product Schedule or proposed by agent vendors for potential use in spill cleanup, it is important that response decision-makers evaluate the various characteristics of agents, particularly their safety and efficacy. From the perspective of planning for bioremediation use, the most appropriate time to evaluate agents whether performed by EPA, product vendors, or contractors - is before a spill occurs. Provided below is a procedure designed specifically to aid in such an evaluation, which is directed ultimately at identifying bioremediation agents that will be safe and effective in field applications. There may be circumstances, however, under which there is not adequate time to perform thorough agent evaluations before a decision regarding bioremediation use must be made. In these instances, the procedure below should be used as a guide to determine whether existing information on individual agents is adequate to support further consideration of their use.

The procedure follows a "tiered" approach (a "Base Tier and four subsequent tiers) whereby bioremediation agent performance data is gathered as a means to predict the safety and efficacy of agent applications in various field settings or habitats where oil spills may occur. The proposed procedure is intended as a standard methodology for assessing the effectiveness and safety of different bioremediation agents. Following the procedure will not assure that a tested agent will be effective in spill cleanup, however, following the procedure should increase the level of confidence that use of an oil spill bioremediation agent will be effective and safe.

### Base Tier -- "Go"/"No Go". Requirements and Information

Information on a bioremediation agent should be collected from the agent vendor and an initial screening of the information performed. Objectives of this screening are to:

- Ensure that the agent is listed on EPA's NCP Product Schedule.

OSE II is on the EPA List under bioremediation sub- category EA, B-53. Category EA/OSE II has already been peer reviewed by 31 scientists with the EPA/NETAC contract for bioremediation protocol development, therefore OSE II has already been determined to meet and passed this as a “go.”

- Obtain basic information on a bioremediation agent's makeup;

Category EA/OSE II ’s basic information is denoted in the Types of Bioremediation and its mode of action above (page 6).

- Ensure satisfaction of minimal regulatory approvals that may be required;

Category EA meets all the minimal regulatory approvals and actually meets the requirement of the Clean Water Act requiring

that a response remove the pollutant from the environment.

- Certify whether the agent contains pathogenic, carcinogenic, or hazardous substances or microorganisms normally considered unacceptable for release into the environment; Category EA has certified this to the US EPA in 1991 in order for OSE II to be part of the US EPA NETAC protocol development. The New Zealand EPA has certified that OSE II does not contain any hazardous chemicals, and does not contain any foreign species. Certification number SOS# 100179 Environmental Protection Authority New Zealand.

Information needed from the agent vendor to perform this initial screening includes the agent's exact chemical and biological makeup as well as formulation characteristics, and proof of the agent's listing on the NCP Product Schedule. The make up is housed by the US EPA, and the listing link is_ http://www.epa.gov/oem/content/ncp/products/oseater.htm_____

**Tier I -- Feasibility Assessment**

Additional vendor information on a bioremediation agent should be collected to support an assessment of whether use of the agent is feasible. The objectives of this tier and assessment are to obtain an understanding of a vendor's capabilities; an agent's availability, contents, and proposed method of use; and an agent's history of use, where applicable. Agent information needed from the vendor to perform this assessment includes the following:

- Application rates and methods;

OSE II is mixed 50 to one with unpolluted water from the area, and this mixture is applied one gallon of the OSE II and water mixture to each gallon of hydrocarbon based material spilled.

- Mode of biodegradation and calculated efficiency;

Sub-category EA mode is category type Enzyme Additive (EA)

As covered, while NRT and RRT guidance addresses the (MC) and (NA) bioremediation types extensively in the 2001 *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands*[5] it does not sufficiently detail the mode of action of *Bioremediation Type EA*.[6]

Below are data to remedy this.

## ENZYMATIC AGENT DEFINITION:

Bio-catalysts designed to enhance the emulsification and/or solubilization of oil to make it more available to microorganisms as a source of food or energy. These agents are generally liquid concentrates, which may be mixed with surfactants and nutrients that are manufactured through fermentation. This type of agent is intended to enhance biodegradation by indigenous microorganisms.

## (EA) TYPE MODE OF ACTION:

**Enzyme Additive mode of action is applicable in open/moving water (fresh, salt and brackish), marsh/estuaries, shoreline and soil environments.** When applied, the non-toxic converters and bio-surfactants in Bioremediation Agent (EA) Type eliminate the classic appearance of an oil spill by emulsifying and solubilizing the molecular hydrocarbon structure and eliminating the adhesion properties of crude oil. This usually takes place within the first 5 - 30 minutes (depending on temperature). The emulsified oil continues to float near the surface thereby eliminating a secondary impact to the water column and seabed.

With the toxicity and adhesion properties eliminated, wildlife that may come in contact with the broken down hydrocarbons they will not become coated in oil and oil adherence to marsh, shorelines, sands, and manmade structures is eliminated. The flammability is eliminated in a short time (depending on temperature) protecting ports, harbors and drilling rigs from the potential explosion hazards associated with fuel spills.

A further action of bioremediation category EA, (there are numerous enzymes contained in the product's matrices) is that the enzymes then attach themselves to the hydrocarbons with the biosurfactants, developing protein binding sites, that act as a catalyst to speed up the bioremediation process by inducing enhanced indigenous bacteria to utilize the detoxified oil/hydrocarbons as a food source. The EA category also contains properties that cause all the constituents to remain in contact with the spilled oil/hydrocarbons in moving waters.

Over the next few days or weeks (again, depending on temperature), non-toxic nutrients in the Enzyme Additive type rapidly colonize indigenous bacteria to large numbers. The colonized bacteria consume the detoxified hydrocarbon emulsion, digesting the spill to $CO_2$ and water, thereby permanently removing the oil/hydrocarbons from the environment and resulting in final water clarification. Without category (EA) assistance, this

natural process may take up to 20 years based on Ixtoc and the Valdez spill studies.

**SHORELINES/MARSHES:**

When a spill has already made land fall or contaminated a marsh, category EA can be applied to lift the spill off the marsh grass (or sandy beaches and shorelines), limiting the time the spill can adversely impact these areas. The use of category EA does not deplete the O2 from water since the spill is held on the surface utilizing predominantly atmospheric O2. With category EA there are no tradeoffs or deleterious effects with this response method.

There is no limited window of opportunity for the application of category EA; it can be used in estuaries, in open (salt) water and, moving fresh water in rivers and soil. It is effective as a first response tool and/or when applied days or months after a spill. Category EA can even be applied to oil that is lying on the seabed floor as long as the product can be brought into contact with the oil which will eventually lift it to the surface returning the seabed to pre-spill conditions.

At the date of this writing, there is only one product on the NCP list that falls under this Bioremediation Agent Type EA classification: (B53-EA-OIL SPILL EATER II), and the efficacy will be different for each type of oil characteristic named from fuel to heavy bunker C oil. The area where the spill is located will effect remediation time, open ocean, shoreline, marsh, estuary, tidal flat, river or stream.The average compete remediation will be between 14 and 35 days at temperature above 40F in the field.

- History of use at previous cleanups;

Category EA has been used in almost every conceivable area where hydrocarbon-based material can be released or spilled. There are a few tests and studies attached as exhibit A (page 49) showing the EPA has used witnessed, demonstrations, tested OSE II and learned of first hand accounts of the use of Category EA/OSE II on hundreds of spills on US navigable waters successfully. Category EA/OSE II has been used on over 23,600 spills as of February 2013 since 1989.

- Chemical properties, fate and persistence, and potential toxicity or bioaccumulation for humans, mammals, and birds based on a review of published literature and chemical databases;

Category EA/OSE II is biodegradable and used as a food source for indigenous bacteria, so there is no adverse fate. Indigenous bacteria colonize the matrices of category EA/OSE II. Once the oil is gone, the food source is gone and they die back to their normal background levels, so

there is no persistence. This also eliminates the potential for bio accumulation. Western Florida University under contract from the US EPA Hap Prichard Gulf Breeze, Florida performed toxicity testing with OSE II and showed their was no toxicological concerns, and this included an open mescosm testing which is a good indicator of how OSE II would work in the field. In fact the field has more water, and more area, therefore the toxicity would even be less. The link to their test information is http://www.nbiap.vt.edu/brarg/brasym95/kavanaugh95.htm. These tests are included in the 18 toxicity test document that can be viewed at: http://osei.us/wp-content/uploads/18-Toxicity-test-with-4-2012-Log0.pdf

- Acute or chronic toxicity to one marine or freshwater fish and invertebrate species selected from US EPA's "Effluent Monitoring Program"; and, where available,

18 toxicity tests some performed by the US EPA and Environment Canada showing OSE II is practically non toxic based on the US EPA scale with the average LC 50 for Category EA/OSE II being above 1900, can be viewed and downloaded at: http://osei.us/wp-content/uploads/18-Toxicity-test-with-4-2012-Log0.pdf

- Effectiveness in enhancing biodegradation over a baseline standard or control demonstrated by descriptions and quantitative analytical results of any laboratory or field studies performed (such as results of gas chromatographic analyses of treated and untreated samples for alkanes and/or aromatics).

The US EPA NCP protocol for getting a product on the NCP list has been carried out in its current form with OSE II three times, and in its 21 day form two times by the EPA/NETAC, University of Texas A&M, BP Strike Team for the DWH Macondo spill, and some performed by the EPA, NETAC, and the Department of Interior. The US EPA RRT VII has performed a GC/MS test, as well as the US Marines in 29 Palms, CA, for which they won a US DOD environmental award. Others cited below have performed GC/MS test with OSE II.

http://www.epa.gov/oem/content/ncp/products/oseater.htm
http://osei.us/technical-library-documents efficacy tests pages 25-28
http://www.nbiap.vt.edu/brarg/brasym95/kavanaugh95.htm
http://www.osei.us/pdf%20files/RRT%20plus%20testing.pdf

http://osei.us/brochures click on US Department of Interior study
BCS Strike force team http://osei.us/pdf%20files/osei_sum.pdf

There are numerous more tests by Universities and Oil companies available upon request

A description of the management structure and qualifications of the vendor's organization is also needed.

OSEI Corporation Business Profile
II. OSEI Corporation, Mailing address
P.O. Box 515429
Dallas, Texas 75251
Phone (972) 669- 3390, answered 24 hours a day, 7 days a week
Fax (469)241- 0896
Email: oseicorp@msn.com
URL www.osei.us

III. Headquarters :
2 story Building 23,000 sq ft under construction starting May 2011
Offices, sales, accounting and executives

IV. Manufacturing facility.
Single story building with 65,000 sq. ft, with rail siding. Manufacturing capacity is
2000, 55 gallon drums, and 100, 5 gallon cases a month. With a 30 days notice,
manufacturing capability can be increased to 8,000, 55 gallon drums and 1,000, 5
gallon cases, with a contingency plan to double this output if needed.
P.O. Box 515429
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Ph: (972) 669- 3390
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Email: oseicorp@msn.com
Web: www.osei.us

P2 OSEI Corporation Company Profile

V. Warehouse
Single story building in DFW Texas 75,000 sq ft with rail siding in which

product can
be air shipped any where in the world 24/7. We normally stock 150 to 200, 55 gallon
drums and 50, 5 gallon cases.

VI. Primary Corporation Contact
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Serena  Sales Rep Gulf  States
Kevin Barteir Sales rep Gulf states
David FaKouri rep Gulf states
Carl McCool rep Gulf states
Eric Hamilton  Florida
Mark Rettig Rep Florida
Randy Hutto Rep Ohio Florida
Jason Patrick Rep  North Carolina, South Carolina,
Jeff Goldware Rep Georgia
Gil Schrock Sales Rep California
T.K. Oshanahan Sales rep Ontario Canada
Alisa Utamating Sales Rep Taiwan
Hassan Sherif Sales rep Saudia Arabia
Mat Garner rep Colorado
Spencer Smith Training
Dennis Marketic Liasion officer between OSEI Corporation and BP
Ha (Harrold) Hong Sang rep China
Masa Kachi Japan, distributor

Larry Casey Rep for Taiwan, China, and Brazil
Luis Miranda  Brazil
David Kim  Wisco South Korea, distributor
Mary Economacou Greece, distributor
Jon Stavros Greece
Antonio Romeo Canada,
Raymond Reesor Toronto Canada
Maol Lesi, Nigeria distributor
Ken Bergstrom Nigeria
Patrick Abachu Lagos Nigeria
Yakubuh Hussaini Ghana distributor
Dr. Saied Azizi UAE, distributor
Abdul Khader UAE
Mr. Atif  M. Al Hassan RMC Company Saudia Arabia distributor
Joel Farhadian Australia
Stan Arceiri Kuwait
Bill Porter Iraq
Mariano Gomez Argentina
Charlie Hobbs New Zealand
Sanjay Chada Qatar
Raghunath Mahabel India
Elie Balas Israel
Ami Amiadini California
Rich Prock Carribean and Virgin Islands
Donna Prock Carribean and Virgin Islands
Holly Hill  Carribean and Virgin Islands

**Tier II - Laboratory-Scale Data**

**Sub-category EA/OSE II has already been tested in Tier II and peer reviewed by 31 scientists that sit on the EPA/NETAC panel and they approved Category EA/OSE II for Tier III testing which demonstrated OSE II does, in fact, remediated oil. The fact that the EPA had Western Florida University perform open mescosm efficacy and toxicity testing, as cited, proves OSE II met or exceeded Tier II and was approved for Tier III.**

Standard laboratory methods should be used to develop data on an agent's toxicity and its ability to stimulate the biodegradation of a standard oil.  The specific

objectives of this tier are to evaluate the relative ability of a bioremediation agent to degrade oil, or stimulate the rate of biodegradation, under defined and controlled laboratory conditions and to determine the potential toxicity associated with the agent's use through the performance of standard toxicity tests. Analytical methods developed by EPA should be used to perform these laboratory studies.

The approach to evaluate an agent's relative effectiveness at degrading oil should:

- Provide sufficient information to indicate with a firm degree of confidence that the agent is degrading oil constituents;
- Provide an indicator of total microbial activity; and
- Assure the viability of the culture being tested, where applicable.

The approach should include temperature, salinity, and nutrient testing to document the conditions under which an agent's ability to degrade a standard type of oil was determined.

The approach to evaluate an agent's toxicity should be conducted for specific fresh-water or marine species on the agent alone and the agent and standardized oil combined. Seven-day chronic estimator methods should be performed using daphnia (Ceriodaphnia) and fathead minnows (Pimephales) for fresh water, and mysids (Mysidopsis) and silversides (Menidia) for marine applications. These are standard tests; additional tests specific for Regional species may be desirable. Mammalian toxicity of agent constituents should be reviewed in existing data to determine whether any precautions need be taken with regard to application methods, rates, or timing to protect persons applying agents as well as indigenous wild life.

This was performed with Category EA/OSE II by EPA through Western Florida University. See link http://www.nbiap.vt.edu/brarg/brasym95/kavanaugh95.htm

See excerpts from the tests. The following findings are discussed:

Seven-day static-renewal toxicity tests with five CBA's indicated that the products have relatively low intrinsic toxicity to mysids and inland silversides (concentrations producing no-observable effects were 75 mg/L).

Application of CBAs to oil in simulated open-water test systems resulted in effluents with no apparent chronic toxicity to *M. bahia* for five of the six products tested. OSE II 's tests showing the LC 50 for all nine tests, including a 7 day test on both species, the LC 50 was 5,000 or greater (non toxic).

**Tier Ill - Simulated Field Test Demonstration**

Sub-category EA/OSE II was successfully tested for toxicity and showed great promise of remediation from the test results provided for simulated open mesocosm testing. The simulated open field testing in Tier III results presented to the OSEI Corporation were promising showing OSE II can be used in open water and shorelines.

Based on findings of previous tiers, microcosm systems should be used to perform simulated field test demonstrations on a bioremediation agent, as appropriate. The objective of this tier is to predict a bioremediation agent's effectiveness at degrading oil or petroleum products in specific field settings or habitats.

Although EPA-approved microcosm systems for performing simulated field test demonstrations are still under development at the time of this writing, the approach for performing these tests is to use microcosm systems that simulate actual biodegradation field kinetics. This approach will aid in determining the relative effectiveness and toxicity of an agent under conditions that cannot be modeled in standard laboratory methods, such as those proposed in Tier 11 of the procedure. Microcosm systems that should be considered for simulated field test demonstrations of agents include:

1. cobble beaches, both marine and fresh water;
2. open water, both marine and fresh, warm and arctic;
3. marshes and wetlands, both marine and fresh water;
4. inland shoreline;
5. sandy beaches, both marine and fresh water; and,
6. land/soil.

**Tier IV -- Limited Field-Scale Demonstration of the Agent**

OSE II was approved or presented for Tier IV testing before the testing was abruptly stopped. OSE II however since 1992 OSE II has significant open water and shoreline clean ups performed in US navigable waters and elsewhere globally. OSE II has also been demonstrated on water and shorelines as well. The need for limited Filed scale demonstration has been surpassed by actual numerous demonstrations and clean ups of open water and shorelines.

Depending on the results of the simulated field test demonstration in Tier III, a limited field scale demonstration of a bioremediation agent should be conducted. The objectives of this field demonstration are to test the effectiveness and toxicity of the bioremediation agent in actual field tests and to verify the accuracy of Tier Ill laboratory results in predicting field efficacy using the actual field monitoring data obtained. The approach for performing these demonstrations is to collect information during active field testing to support an evaluation to confirm the bioremediation agent's estimated

environmental safety and efficacy.

At this time, EPA-approved protocols for performing limited field-scale demonstrations in various settings are still under development. Until such protocols become available, the guidelines provided in Section 6 for monitoring field applications of bioremediation agents could be used for evaluating limited field-scale demonstrations of agents.

Category EA has been used extensively, and tested extensively, and after 23 years has proven to be a tried and trued safe effective first response bioremediation clean up tool that has numerous associations through testing and successful clean ups with US EPA/RRT's. See attached exhibit A

**Agent Selection**

Due to a lack of specific bioremediation agent research and agent testing standards in the US, except with Sub-category EA/OSE II the selection of a bioremediation agent that will enhance the rate of oil biodegradation must be based on best professional judgment. For most of the bioremediation agents currently on the NCP Product Schedule, there are only limited comparative data by which to measure their relative efficacy and safety. Some of the agents have been tested by EPA according to the procedure described above; however, these agents are not necessarily better than ones that have not been tested by these methods. Therefore, agent selection will remain largely a subjective process until a larger and more complete database of standard test data on agents can be assembled.

To the extent possible, the selection of bioremediation agents for potential use in oil spill cleanup against specific oils or petroleum products should take place in anticipation of an oil discharge, when time is not a critical factor. For areas where the potential for an accidental spill is high or where there has been a high frequency of spills (assuming the use of bioremediation agents is allowed in these areas), specific plans should be developed that outline the most likely petroleum products to be spilled and the alternative bioremediation agents that could be used to perform cleanup of those products in these areas.

The US Coast Guard has an extensive track record of use of OSE II (Coast Guard link http://www.osei.us/pdf%20files/Coast%20Guard%20BP%20spill%20approval%201.pdf.
Coast Guard Commandant Paul Yost requested the responsible party of the Valdez spill to test OSE II. Exxon tested OSE II in the winter of 1990 in Florham Park, New Jersey along with at least 10 other products Exxon thought were the best products in the world, including the toxic Exxon product called Inipol. Dr. Brown of the University of Alaska witnessed the

test and relayed to us that OSE II was 92% more effective than the next best product. Despite this, Exxon continued to use Inipol.

The US Navy used OSE II on US navigable waters spills in San Diego Bay on hundreds of spills, with whales and dolphins around without any adverse effects to any marine species while reducing their clean up cost over 87%. The US EPA Debra Dietrich and Nick Nichols met with the Navy officials with the OSEI Corporation in San Diego where EPA officials learned about the 100's of cleanups performed by the US Navy for 3½ years.
Opening link to OSEI home page http://osei.us/

The US EPA has extensively tested and authorized through a contractor the use of OSE II on the Osage Indian reservation oil spill on US navigable waters, after trying numerous other methods and products over a 1½ year period with no success. Once OSE II was utilized, in 45 days the spill was cleaned up to the test level of "non detect."
http://osei.us/photoalbums/osage-indian-reservation-epa-cleanup

EPA RRT IV officials witnessed a successful demonstration of OSE II on the beach in Waveland, Mississippi under direction of Senator Tommy Gollot. See link http://osei.us/archives/819.

The Department of Interior recently successful tested OSE II and it out performed the historical method of mechanical cleanup and it out performed both Corexit 9500 and 9527 since they only sink oil and do not remove oil from the environment. During the DOI test, the two Corexits were not even effective at sinking the oil. The mechanical clean up achieved it's historical norm of cleaning up between 2 to 8% of the total oil. OSE II was proven by DOI to be the superior clean up method. This RRT trustee's test proves what is the most effective clean up response that meets the Clean Water Act requirements of permanently removing oil from the environment. See test summary at link http://osei.us/brochures - click on US Department of Interior study, where the summary of the test can be viewed, as well.
http://www.google.com/search?client=safari&rls=en&q=OSEI+summary+of+Department+of+interior+test&ie=UTF-8&oe=UTF-8

DOC and NOAA officials have also witnessed a successful demonstration of OSE II at Mo Hang Port, South Korea. See link pictures 46 and 47

http://osei.us/photoalbums/south-korea-hebie-spirit-2, where they saw the effectiveness of OSE II on Bunker C oil.
US NOAA officials visited a demonstration of OSE II, in Mo Hang Port South Korea. In the pictures at the following link, the gentleman in the yellow jacket was the head of the South Korean Coast Guard. He explained the very successful testing of OSE II, which led to a successful demonstration on the shoreline with South Korean government officials and the approval of OSE II for South Korea, as well. These are pictures of NOAA officials wearing NOAA caps at the demonstration. The conclusion of the successful test showed OSE II remediating the Bunker C oil to CO2 and water, and showed there were small crabs that were living in the water for the duration of the test unharmed. See link http://osei.us/photoalbums/south-korea-hebie-spirit-2 - scroll to the bottom of the set of pictures to see the NOAA officials.

NOAA official Charlie Henry letter. See link http://www.osei.us/pdf%20files/NOAA%20Charlie%20Henry%20final%201%2025%202011%20.pdf

Also see Department of Energy use of OSE II http://osei.us/photoalbums/department-of-energy-use-of-ose-ii-2

**Demonstrations on water and shorelines**

The four main Federal groups associated with the RRT's and their decisions for what to pre approve and use on spills all have first-hand use or witnessing of the use of OSE II being applied to water and shorelines successfully. The category Sub-category EA/OSE II, as can be seen through videos, pictures and test results for efficacy and toxicity is the safest for humans, non-toxic to marine species, tried and true means to permanently remove oil/hydrocarbon-based material from the environment. The Compilation of Documentation and Use of OSEII with RRT is Exhibit A (page 49). That document contains a statement from EPA RRT VI that they cannot find a scientific reason why not to use OSE II. It is time for the US EPA, Coast Guard, DOI, DOC and the rest of the federal agencies to use what the Clean Water Act intended and that is a safe means to permanently remove oil from the environment.

## APPENDIX E

## LABORATORY ANALYSIS PARAMETERS

| Parameter | Sample Matrix | Methodology | Recommended Methods |
|---|---|---|---|
| Oil hydrocarbons (C17, pristane, C18, ne) | Water, Sediment or shore ial | GC + GC/MS | ASTM Method D3328 |
| $NH_3$ | oreline material | Spectrophotometric | EPA Method 350.1, 350.2 c |
| $NO_3$ | Water, Sediment or shore ial | Spectrophotometric | EPA Method 353.2 or 353.3 |
| $NO_2$ | Water, Sediment or shore ial | Spectrophotometric | EPA Method 354.1 |
| $PO_4$ | Water, Sediment or shore ial | Spectrophotometric | EPA Method 365.1, 365.2 c |
| Toxicity | Water, Sediment or shore ial | | |

Sampling is to be conducted in accordance with an approved sampling plan and should utilize a justified random approach where the individual sites are selected based on appropriate habitat-types within treated and untreated zones. Within a site, individual sampling stations should be randomly chosen. Dependent on habitat-type, the site may be further divided such that specific zones within the site are monitored such as the upper and lower intertidal zones or stream-side and back marsh areas. Sediment grab samples may be collected using a variety of standard techniques. Core sampling is preferred for most intertidal and subtidal areas since it consistently allows for a highly reproducible volume of sample to be collected. Typically the core depth should exceed the depth of contamination if applicable and the core should be sectioned by 5 cm increments. Scoop-type grab sampling is applicable but great care is required to ensure that consistency is maintained. The sampling plan should provide exact guidance as to the width and depth of each sample.

Adjacent subsurface water samples may be collected using standard grab techniques. Caution should be exercised to prevent surface oil from contaminating the collection vessel as it is lowered to the specified sampling depth. Water grab sample will typically be collected at 1-3'depth.

Analytical methods used for bioremediation monitoring should be consistent with standard methods utilized for oil weathering and degradation studies. Analytical guidance being developed by the EPA and NETAC for laboratory testing of

bioremediation agents should be adopted for field monitoring studies.

Field and laboratory blanks should be specified in the monitoring plan and should represent at least 10% of the samples analyzed. To assess environmental variability, 10% of the sample stations should be sampled and analyzed in triplicate. Since no certified reference material is currently available for oil bioremediation monitoring, a reference sample of the spilled oil should be analyzed periodically to verify laboratory consistency. Quantitative values for the reference oil should not vary by more than 20% for selected analytes. Good laboratory practices should be employed that are consistent with the objectives of the biomonitoring plan.

Accurate sample identification and proper control of samples is essential. A chain of custody procedure will be established and implemented which will ensure integrity of the samples and proper handling of the samples.

## APPENDIX F

## INFORMATION FEEDBACK:
## BIOREMEDIATION USE FOLLOW-UP FORM

Lessons learned from a spill cleanup operation are most useful when others, particularly those not personally involved in the original cleanup operation, can benefit from them by drawing upon the original responders' experiences. Region 4 has established a program to facilitate the collection and transfer of information on uses of bioremediation that is intended to provide decision makers with case data upon which future decisions regarding bioremediation may be based. Particularly because response officials have very limited experience with bioremediation in uncontrolled environments, such as open water and other marine areas, this program is expected to be a valuable resource for supporting informed decisions regarding bioremediation.

The principal objective of this bioremediation information feedback program in Region 4 are as follows:

To gather relevant, accurate, descriptive, and complete information from sites - where bioremediation has been used for spill response; and

To provide that information via an accessible network to future decision makers who are considering the use of bioremediation.

The Bioremediation Use Follow-Up Form on the following pages has been provided to guide information collection efforts in support of this program. A separate form should be completed for each unique bioremediation activity. Because certain information may not have been anticipated when the form was developed, feel free to provide any other information deemed appropriate regarding the use of bioremediation in a particular response action.

### BIOREMEDIATION USE FOLLOW-UP FORM

A. <u>SPILL INFORMATION</u>

1. Spill event
2. Date
3. Location (e.g., offshore, wetlands, coastal)
4. Product(s) spilled
5. Amount of spill
6. Reason(s) for using bioremediation
7. Age of oil when bioremediation agents applied

B. <u>BIOREMEDIATION AGENT INFORMATION</u>

1 . First Treatment or Application:

a. Type of agent applied (e.g., nutrient, microbial, enzyme)
b. Name of agent
c. Agent listed on the NCP Product Schedule?
d. Vendor
e. Vendor address and phone number
f. Rate effectiveness (compared to control site) on a scale of 1 to 10, 10 being the highest score

Visual observation
Oil chemistry
Method used (e.g., GC, GC/MS, TPH)

2. Second Treatment or Application (complete if different from above):

a. Type of agent applied (e.g., nutrient, microbial, enzyme)
b. Name of agent
c. Agent listed on the NCP Product Schedule?
d. Vendor
e. Vendor address and phone number
f. Rate effectiveness (compared to control site) on a scale of 1 to 10, 10 being the highest score

Visual observation
Oil chemistry
Method used (e.g., GC, GC/MS, TPH)

3. Third Treatment or Application (complete if different from above):

a. Type of agent applied (e.g., nutrient, microbial, enzyme)
b. Name of agent
C. Agent listed on the NCP Product Schedule?
d. Vendor
e. Vendor address and phone number
f. Rate effectiveness (compared to control site) on a scale of 1 to 10, 10 being the highest score

Visual observation
Oil chemistry
Method used (e.g., GC, GC/MS, TPH)

C. <u>SITE CONTROLS</u>

1. Size and number of test site(s)
2. Size and number of control site(s)
3. Site security measures taken

D. TREATMENT AREA LOCATION

1 . On water (latitude and longitude)
2. Shoreline (latitude and longitude)
   Shoreline type (e.g., sand, shell, cobble)
   Shoreline zone (e.g., intertidal, surge, storm/overwash) Depth of shoreline oiling

E. APPLICATION INFORMATION

1. Microbial counts before application
2. Microbial counts after application
3. Applications performed by (names and titles)
4. Application method(s) used
5. Application date(s)
6. Application conditions (e.g., winds, waves)
7. Agent concentration and rates (e.g., gal/acre)
8. Additional information on re-applications

F. MONITORING

1 . Schedule and duration (e.g., weekly for 3 months)
2. Method (e.g., foot, by air, boat)
3. Monitoring performed by (names and titles)
4. Toxicity noted

G. PROBLEMS ENCOUNTERED (e.g., weather, site security, application)

H. LESSONS LEARNED

1. CONTACTS
   1. OSC (name, address, and phone)
   2. SSC (name, address, and phone)
   3. Form completed by (name, title, and agency)

## APPENDIX G

## REFERENCES

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[i] Bioremediation [Types MC and NA] for 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. [with the exception of NCP Listed Type EA based on extensive field use and testing on fresh and weathered hydrocarbons/oil. It recently demonstrated an 80% rate of PAH degradation on Macondo Block LA sweet crude containing Corexit per March 3, 2011- BP BCST D.Tsao , LSU R..J. Portier, L. M. Basirico *Laboratory Screening of Commercial Bioremediation Agents for the Deepwater Horizon Spill Response.]*

[ii] 2001 *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands* (http://www.epa.gov/osweroe1/docs/oil/edu/bioremed.pdf)

[iii] This description of the EA Type mode of action is based on the NCP listed sole sourced product Oil Spill Eater II's field use and test documentation on fresh and weathered hydrocarbons/oil in ocean, fresh water and shoreline environments. If another EA Type product is added to the NCP List, these descriptions may not apply and should be validated in field tests with that product.

[iv] As per NRT Science and Technology Committee Bioremediation Fact Sheet: "Added bacteria seem to compete poorly with the indigenous population." … "and has not been shown to have any long-term beneficial effects in shoreline cleanup"

## Further References:


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U.S. Environmental Protection Agency Office of Research and Development National Risk Management Research Laboratory Land Remediation and Pollution Control Division 26 W. Martin Luther King Drive Cincinnati, OH--added bacteria seem to compete poorly with the indigenous population (Tagger et al., 1983; Lee and Levy, 1989; Venosa et al., 1992)--biostimulation alone had a greater effect on oil biodegradation than the microbial seeding (Jobson et al., 1974; Lee and Levy, 1987; Lee et al., 1997b, Venosa et al., 1996).


## Bioremediation Sub-Category EA Type References:


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Galen Bartman, *Oil Spill Eater Respirocity Evaluation CAI Lab. No. 3265* (July 1990) additive [EA] has a meaningful and significant effect on decreasing the oil concentration and increasing the oxygen take up.

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(Oct 93) pg 66 http://osei.us/tech-library-pdfs/2011/OSEI%20Manual_FINAL-2011.pdf
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Environmental Protection Authority New Zealand, Hazardous Substances Division, Benjamin Sowman, (16 July 2012); SOS # 1001797; Determination of the Status of Oil Spill Eater II-Non Hazardous

2001 *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands* (http://www.epa.gov/osweroe1/docs/oil/edu/bioremed.pdf)

This description of the EA Type mode of action is based on the NCP-listed, Oil Spill Eater II's field use and test documentation on fresh and weathered hydrocarbons/oil in ocean, fresh water and shoreline environments. At this time, OSE II is accurately categorized as a sole-sourced product by official government procurement legal standards. If another sub-category EA Type product is added to the NCP List, these descriptions may not apply and should be validated in field tests with that specific product.