

**SAFETY DATA SHEET****PRODUCT****COREXIT(R) EC9527A****EMERGENCY TELEPHONE NUMBER(S)****(800) 424-9300 (24 Hours) CHEMTREC****1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION****PRODUCT NAME :** COREXIT(R) EC9527A**APPLICATION :** OIL SPILL DISPERSANT**COMPANY IDENTIFICATION :** Nalco Company
1601 W. Diehl Road
Naperville, Illinois
60563-1198**EMERGENCY TELEPHONE NUMBER(S) :** (800) 424-9300 (24 Hours) CHEMTREC**NFPA 704M/HMIS RATING****HEALTH :** 2/2 **FLAMMABILITY :** 1/1 **INSTABILITY :** 0/0 **OTHER :**

0 = Insignificant 1 = Slight 2 = Moderate 3 = High 4 = Extreme

2. COMPOSITION/INFORMATION ON INGREDIENTS

Our hazard evaluation has identified the following chemical substance(s) as hazardous. Consult Section 15 for the nature of the hazard(s).

Hazardous Substance(s)	CAS NO	% (w/w)
2-Butoxyethanol	111-76-2	30.0 - 60.0
Organic sulfonic acid salt	Proprietary	10.0 - 30.0
Propylene Glycol	57-55-6	1.0 - 5.0

3. HAZARDS IDENTIFICATION****EMERGENCY OVERVIEW******WARNING**

Eye and skin irritant. Repeated or excessive exposure to butoxyethanol may cause injury to red blood cells (hemolysis), kidney or the liver. Harmful by inhalation, in contact with skin and if swallowed.

Do not get in eyes, on skin, on clothing. Do not take internally. Use with adequate ventilation. Wear suitable protective clothing. Keep container tightly closed. Flush affected area with water. Keep away from heat. Keep away from sources of ignition - No smoking.

May evolve oxides of carbon (COx) under fire conditions.

PRIMARY ROUTES OF EXPOSURE :

Eye, Skin

HUMAN HEALTH HAZARDS - ACUTE :**EYE CONTACT :**

Can cause moderate irritation.

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SKIN CONTACT :

Can cause moderate irritation. Harmful if absorbed through skin.

INGESTION :

May be harmful if swallowed. May cause liver and kidney effects and/or damage. There may be irritation to the gastro-intestinal tract.

INHALATION :

Harmful by inhalation. Repeated or prolonged exposure may irritate the respiratory tract.

SYMPTOMS OF EXPOSURE :

Acute :

Excessive exposure may cause central nervous system effects, nausea, vomiting, anesthetic or narcotic effects.

Chronic :

Repeated or excessive exposure to butoxyethanol may cause injury to red blood cells (hemolysis), kidney or the liver.

AGGRAVATION OF EXISTING CONDITIONS :

Skin contact may aggravate an existing dermatitis condition.

HUMAN HEALTH HAZARDS - CHRONIC :

Contains ethylene glycol monobutyl ether (butoxyethanol). Prolonged and/or repeated exposure through inhalation or extensive skin contact with EGBE may result in damage to the blood and kidneys.

4. FIRST AID MEASURES

EYE CONTACT :

Flush affected area with water. Get medical attention.

SKIN CONTACT :

Flush affected area with water. Get medical attention.

INGESTION :

Do not induce vomiting without medical advice. If conscious, washout mouth and give water to drink. Get medical attention.

INHALATION :

Remove to fresh air, treat symptomatically. If symptoms develop, seek medical advice.

NOTE TO PHYSICIAN :

Based on the individual reactions of the patient, the physician's judgement should be used to control symptoms and clinical condition.

5. FIRE FIGHTING MEASURES

FLASH POINT : 163 °F / 72.7 °C (TCC)

This product does not sustain combustion per the method outlined in 49 CFR Appendix H.

**SAFETY DATA SHEET****PRODUCT****COREXIT(R) EC9527A****EMERGENCY TELEPHONE NUMBER(S)****(800) 424-9300 (24 Hours) CHEMTREC****EXTINGUISHING MEDIA :**

This product would not be expected to burn unless all the water is boiled away. The remaining organics may be ignitable. Use extinguishing media appropriate for surrounding fire.

FIRE AND EXPLOSION HAZARD :

May evolve oxides of carbon (COx) under fire conditions.

SPECIAL PROTECTIVE EQUIPMENT FOR FIRE FIGHTING :

In case of fire, wear a full face positive-pressure self contained breathing apparatus and protective suit.

6. ACCIDENTAL RELEASE MEASURES**PERSONAL PRECAUTIONS :**

Restrict access to area as appropriate until clean-up operations are complete. Stop or reduce any leaks if it is safe to do so. Do not touch spilled material. Ventilate spill area if possible. Use personal protective equipment recommended in Section 8 (Exposure Controls/Personal Protection).

METHODS FOR CLEANING UP :

SMALL SPILLS: Soak up spill with absorbent material. Place residues in a suitable, covered, properly labeled container. Wash affected area. **LARGE SPILLS:** Contain liquid using absorbent material, by digging trenches or by diking. Reclaim into recovery or salvage drums or tank truck for proper disposal. Contact an approved waste hauler for disposal of contaminated recovered material. Dispose of material in compliance with regulations indicated in Section 13 (Disposal Considerations).

ENVIRONMENTAL PRECAUTIONS :

Do not contaminate surface water.

7. HANDLING AND STORAGE**HANDLING :**

Avoid eye and skin contact. Do not take internally. Ensure all containers are labeled. Keep the containers closed when not in use.

STORAGE CONDITIONS :

Store the containers tightly closed.

SUITABLE CONSTRUCTION MATERIAL :

Stainless Steel 316L, Hastelloy C-276, MDPE (medium density polyethylene), Nitrile, Plexiglass, Kalrez, TFE, Alfax, Teflon, HDPE (high density polyethylene), Neoprene, Aluminum, Polypropylene, Polyethylene, Carbon Steel C1018, Stainless Steel 304, Compatibility with Plastic Materials can vary; we therefore recommend that compatibility is tested prior to use., FEP (encapsulated), Perfluoroelastomer, PVC

UNSUITABLE CONSTRUCTION MATERIAL :

Copper, Mild steel, Brass, Nylon, Buna-N, Natural rubber, Polyurethane, Hypalon, Viton, Ethylene propylene, EPDM

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Exposure guidelines have not been established for this product. Available exposure limits for the substance(s) are shown below.

ACGIH/TLV :

Substance(s)

2-Butoxyethanol TWA: 20 ppm , 97 mg/m³

Propylene Glycol

OSHA/PEL :

Substance(s)

2-Butoxyethanol TWA: 50 ppm , 240 mg/m³ (Skin)

Propylene Glycol

AIHA/WEEL :

Substance(s)

For propylene glycol, an 8 hour TWA of 10 mg/m³ (aerosol) and 50 ppm (total).**ENGINEERING MEASURES :**

General ventilation is recommended.

RESPIRATORY PROTECTION :

Where concentrations in air may exceed the limits given in this section, the use of a half face filter mask or air supplied breathing apparatus is recommended. A suitable filter material depends on the amount and type of chemicals being handled. Consider the use of filter type: Multi-contaminant cartridge, with a Particulate pre-filter. In event of emergency or planned entry into unknown concentrations a positive pressure, full-facepiece SCBA should be used. If respiratory protection is required, institute a complete respiratory protection program including selection, fit testing, training, maintenance and inspection.

HAND PROTECTION :

Neoprene gloves, Nitrile gloves, Butyl gloves, PVC gloves

SKIN PROTECTION :

Wear standard protective clothing.

EYE PROTECTION :

Wear chemical splash goggles.

HYGIENE RECOMMENDATIONS :

Keep an eye wash fountain available. Keep a safety shower available. If clothing is contaminated, remove clothing and thoroughly wash the affected area. Launder contaminated clothing before reuse.

HUMAN EXPOSURE CHARACTERIZATION :

Based on our recommended product application and personal protective equipment, the potential human exposure is: Low

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9. PHYSICAL AND CHEMICAL PROPERTIES

PHYSICAL STATE	Liquid
APPEARANCE	Clear Amber
ODOR	Mild
SPECIFIC GRAVITY	0.98 - 1.02
DENSITY	8.2 - 8.5 lb/gal
SOLUBILITY IN WATER	Complete
pH (100 %)	6.1
VISCOSITY	160 cst @ 32 °F / 0 °C
POUR POINT	ASTM D-97 -66.9 °F / -55 °C
POUR POINT	< -40 °F / < -40 °C
BOILING POINT	340 °F / 171 °C
VAPOR PRESSURE	< 5 mm Hg @ 100 °F / 38 °C Same as water
EVAPORATION RATE	0.1

Note: These physical properties are typical values for this product and are subject to change.

10. STABILITY AND REACTIVITY**STABILITY :**

Stable under normal conditions.

HAZARDOUS POLYMERIZATION :

Hazardous polymerization will not occur.

CONDITIONS TO AVOID :

Extremes of temperature

MATERIALS TO AVOID :

Contact with strong oxidizers (e.g. chlorine, peroxides, chromates, nitric acid, perchlorate, concentrated oxygen, permanganate) may generate heat, fires, explosions and/or toxic vapors.

HAZARDOUS DECOMPOSITION PRODUCTS :

Under fire conditions: Oxides of carbon

11. TOXICOLOGICAL INFORMATION

No toxicity studies have been conducted on this product.

SENSITIZATION :

This product is not expected to be a sensitizer.

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CARCINOGENICITY :

None of the substances in this product are listed as carcinogens by the International Agency for Research on Cancer (IARC), the National Toxicology Program (NTP) or the American Conference of Governmental Industrial Hygienists (ACGIH).

HUMAN HAZARD CHARACTERIZATION :

Based on our hazard characterization, the potential human hazard is: High

12. ECOLOGICAL INFORMATION**ECOTOXICOLOGICAL EFFECTS :**

No toxicity studies have been conducted on this product.

ACUTE FISH RESULTS :

Species	Exposure	LC50	Test Descriptor
Turbot	96 hrs	50 mg/l	

MOBILITY :

The environmental fate was estimated using a level III fugacity model embedded in the EPI (estimation program interface) Suite TM, provided by the US EPA. The model assumes a steady state condition between the total input and output. The level III model does not require equilibrium between the defined media. The information provided is intended to give the user a general estimate of the environmental fate of this product under the defined conditions of the models.

If released into the environment this material is expected to distribute to the air, water and soil/sediment in the approximate respective percentages;

Air	Water	Soil/Sediment
<5%	10 - 30%	70 - 90%

The portion in water is expected to be soluble or dispersible.

BIOACCUMULATION POTENTIAL

Component substances have a low potential to bioconcentrate.

ENVIRONMENTAL HAZARD AND EXPOSURE CHARACTERIZATION

Based on our hazard characterization, the potential environmental hazard is: Moderate

Based on our recommended product application and the product's characteristics, the potential environmental exposure is: Low

If released into the environment, see CERCLA/SUPERFUND in Section 15.

13. DISPOSAL CONSIDERATIONS

If this product becomes a waste, it is not a hazardous waste as defined by the Resource Conservation and Recovery Act (RCRA) 40 CFR 261, since it does not have the characteristics of Subpart C, nor is it listed under Subpart D.

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As a non-hazardous waste, it is not subject to federal regulation. Consult state or local regulation for any additional handling, treatment or disposal requirements. For disposal, contact a properly licensed waste treatment, storage, disposal or recycling facility.

14. TRANSPORT INFORMATION

The information in this section is for reference only and should not take the place of a shipping paper (bill of lading) specific to an order. Please note that the proper Shipping Name / Hazard Class may vary by packaging, properties, and mode of transportation. Typical Proper Shipping Names for this product are as follows.

LAND TRANSPORT :

Proper Shipping Name : PRODUCT IS NOT REGULATED DURING TRANSPORTATION

AIR TRANSPORT (ICAO/IATA) :

Proper Shipping Name : PRODUCT IS NOT REGULATED DURING TRANSPORTATION

MARINE TRANSPORT (IMDG/IMO) :

Proper Shipping Name : PRODUCT IS NOT REGULATED DURING TRANSPORTATION

15. REGULATORY INFORMATION

This section contains additional information that may have relevance to regulatory compliance. The information in this section is for reference only. It is not exhaustive, and should not be relied upon to take the place of an individualized compliance or hazard assessment. Nalco accepts no liability for the use of this information.

NATIONAL REGULATIONS, USA :**OSHA HAZARD COMMUNICATION RULE, 29 CFR 1910.1200 :**

Based on our hazard evaluation, none of the substances in this product are hazardous.

CERCLA/SUPERFUND, 40 CFR 117, 302 :

Notification of spills of this product is not required.

SARA/SUPERFUND AMENDMENTS AND REAUTHORIZATION ACT OF 1986 (TITLE III) - SECTIONS 302, 311, 312, AND 313 :**SECTION 302 - EXTREMELY HAZARDOUS SUBSTANCES (40 CFR 355) :**

This product does not contain substances listed in Appendix A and B as an Extremely Hazardous Substance.

**SAFETY DATA SHEET****PRODUCT****COREXIT(R) EC9527A****EMERGENCY TELEPHONE NUMBER(S)****(800) 424-9300 (24 Hours) CHEMTREC****SECTIONS 311 AND 312 - MATERIAL SAFETY DATA SHEET REQUIREMENTS (40 CFR 370) :**

Our hazard evaluation has found this product to be hazardous. The product should be reported under the following indicated EPA hazard categories:

- X Immediate (Acute) Health Hazard
- X Delayed (Chronic) Health Hazard
- X Fire Hazard
- X Sudden Release of Pressure Hazard
- X Reactive Hazard

Under SARA 311 and 312, the EPA has established threshold quantities for the reporting of hazardous chemicals. The current thresholds are: 500 pounds or the threshold planning quantity (TPQ), whichever is lower, for extremely hazardous substances and 10,000 pounds for all other hazardous chemicals.

SECTION 313 - LIST OF TOXIC CHEMICALS (40 CFR 372) :

This product contains the following substance(s), (with CAS # and % range) which appear(s) on the List of Toxic Chemicals

<u>Hazardous Substance(s)</u>	<u>CAS NO</u>	<u>% (w/w)</u>
Glycol Ethers		30 - 60

TOXIC SUBSTANCES CONTROL ACT (TSCA) :

The substances in this preparation are included on or exempted from the TSCA 8(b) Inventory (40 CFR 710)

FEDERAL WATER POLLUTION CONTROL ACT, CLEAN WATER ACT, 40 CFR 401.15 / formerly Sec. 307, 40 CFR 116.4 / formerly Sec. 311 :

None of the substances are specifically listed in the regulation.

CLEAN AIR ACT, Sec. 112 (40 CFR 61, Hazardous Air Pollutants), Sec. 602 (40 CFR 82, Class I and II Ozone Depleting Substances) :

None of the substances are specifically listed in the regulation.

CALIFORNIA PROPOSITION 65 :

This product does not contain substances which require warning under California Proposition 65.

MICHIGAN CRITICAL MATERIALS :

None of the substances are specifically listed in the regulation.

STATE RIGHT TO KNOW LAWS :

The following substances are disclosed for compliance with State Right to Know Laws:

2-Butoxyethanol	111-76-2
Propylene Glycol	57-55-6

NATIONAL REGULATIONS, CANADA :

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WORKPLACE HAZARDOUS MATERIALS INFORMATION SYSTEM (WHMIS) :

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

WHMIS CLASSIFICATION :

D2B - Materials Causing Other Toxic Effects - Toxic Material

CANADIAN ENVIRONMENTAL PROTECTION ACT (CEPA) :

The substances in this preparation are listed on the Domestic Substances List (DSL), are exempt, or have been reported in accordance with the New Substances Notification Regulations.

AUSTRALIA

All substances in this product comply with the National Industrial Chemicals Notification & Assessment Scheme (NICNAS).

CHINA

All substances in this product comply with the Chemical Control Law and are listed on the Inventory of Existing Chemical Substances China (IECSC).

EUROPE

The substance(s) in this preparation are included in or exempted from the EINECS or ELINCS inventories

JAPAN

All substances in this product comply with the Law Regulating the Manufacture and Importation Of Chemical Substances and are listed on the Ministry of International Trade & Industry List (MITI).

KOREA

All substances in this product comply with the Toxic Chemical Control Law (TCCL) and are listed on the Existing Chemicals List (ECL)

PHILIPPINES

All substances in this product comply with the Republic Act 6969 (RA 6969) and are listed on the Philippines Inventory of Chemicals & Chemical Substances (PICCS).

16. OTHER INFORMATION

Due to our commitment to Product Stewardship, we have evaluated the human and environmental hazards and exposures of this product. Based on our recommended use of this product, we have characterized the product's general risk. This information should provide assistance for your own risk management practices. We have evaluated our product's risk as follows:

* The human risk is: Low

* The environmental risk is: Low

Any use inconsistent with our recommendations may affect the risk characterization. Our sales representative will assist you to determine if your product application is consistent with our recommendations. Together we can implement an appropriate risk management process.

**SAFETY DATA SHEET****PRODUCT****COREXIT(R) EC9527A****EMERGENCY TELEPHONE NUMBER(S)****(800) 424-9300 (24 Hours) CHEMTREC**

This product material safety data sheet provides health and safety information. The product is to be used in applications consistent with our product literature. Individuals handling this product should be informed of the recommended safety precautions and should have access to this information. For any other uses, exposures should be evaluated so that appropriate handling practices and training programs can be established to insure safe workplace operations. Please consult your local sales representative for any further information.

REFERENCES

Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices, American Conference of Governmental Industrial Hygienists, OH., (Ariel Insight™™ CD-ROM Version), Ariel Research Corp., Bethesda, MD.

Hazardous Substances Data Bank, National Library of Medicine, Bethesda, Maryland (TOMES CPS™™ CD-ROM Version), Micromedex, Inc., Englewood, CO.

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Geneva: World Health Organization, International Agency for Research on Cancer.

Integrated Risk Information System, U.S. Environmental Protection Agency, Washington, D.C. (TOMES CPS™™ CD-ROM Version), Micromedex, Inc., Englewood, CO.

Annual Report on Carcinogens, National Toxicology Program, U.S. Department of Health and Human Services, Public Health Service.

Title 29 Code of Federal Regulations, Part 1910, Subpart Z, Toxic and Hazardous Substances, Occupational Safety and Health Administration (OSHA), (Ariel Insight™™ CD-ROM Version), Ariel Research Corp., Bethesda, MD.

Registry of Toxic Effects of Chemical Substances, National Institute for Occupational Safety and Health, Cincinnati, OH, (TOMES CPS™™ CD-ROM Version), Micromedex, Inc., Englewood, CO.

Ariel Insight™™ (An integrated guide to industrial chemicals covered under major regulatory and advisory programs), North American Module, Western European Module, Chemical Inventories Module and the Generics Module (Ariel Insight™™ CD-ROM Version), Ariel Research Corp., Bethesda, MD.

The Teratogen Information System, University of Washington, Seattle, WA (TOMES CPS™™ CD-ROM Version), Micromedex, Inc., Englewood, CO.

Prepared By : Product Safety Department
Date issued : 10/15/2008
Version Number : 1.7

OFFICE OF INCIDENT MANAGEMENT & PREPAREDNESS

ISSUE PAPER

SUBJECT: COREXIT 9500 AND 9527 MSDS SUMMARY

COMPANY INFORMATION

COREXIT 9500 and 9527 are produced by Nalco Energy Services, L.P. in Sugar Land, Texas.

PRODUCT INFORMATION: COREXIT 9500

It is classified as an oil dispersant, and is included on the EPA, NCP product schedule. COREXIT 9500 is low-flammability and non-hazardous. Storage parameters are: maximum temp 170°F; minimum temp -30°F. It is a surfactant with an oleophilic solvent delivery system, and should be applied as droplets, not mist, from aircraft or vessels. When being applied by aircraft the optimal altitude is between 30 and 50 feet, but is entirely dependent upon the application equipment, weather, and aircraft. A dispersant to oil ratio of 1:50 to 1:10 is recommended, but varies depending on the type of oil, degree of weathering, temperature, and thickness of the slick. As with all dispersants, timely application ensures the highest degree of success.

TOXICITY

Human

Toxicity tests have not been conducted on humans; however it is not considered a carcinogen by the International Agency for Research on Cancer, the National Toxicology Program, or the American Conference of Governmental Industrial Hygienists. First aid measures that should be taken if exposure occurs include flushing the eyes with water, wash skin with soap and water, and removing the victim to fresh air. For ingestion or inhalation the victim should seek medical attention. The human hazard characterization is moderate.

Ecological

Based on the recommended product application and the product's characteristics the potential environmental exposure is low. COREXIT 9500 component substances have a potential to bioconcentrate.

EFFECTIVENESS

The dispersant effectiveness was conducted using the swirling flask test with South Louisiana (S/L) and Prudhoe Bay (P/B) crude oils. It was found to be 54.7% effective on S/L and 45.3% effective on P/B.

PHYSICAL PROPERTIES

1. Flash Point: 181.4°F
2. Pour Point: Less than -71°F
3. Viscosity: 22.5 cst at 104°F
4. Specific Gravity: 0.949 at 60°F
5. pH: 6.2
6. Chemical Name and Percentage by Weight of the Total Formulation: CONFIDENTIAL
7. Surface Active Agents: CONFIDENTIAL
8. Solvents: CONFIDENTIAL
9. Additives: None
10. Solubility: Miscible

PRODUCT INFORMATION: COREXIT 9527

It is classified as an oil dispersant, and is included on the EPA, NCP product schedule. COREXIT 9527 is non-flammable and non-hazardous. Storage parameters are: maximum temp 170°F; minimum temp -30°F. It is a surfactant with an oleophilic solvent delivery system, and should be applied as droplets, not mist, from aircraft or vessels. When being applied by aircraft the optimal altitude is between 30 and 50 feet, but is entirely dependent upon the application equipment, weather, and aircraft. A dispersant to oil ratio of 1:50 to 1:10 is recommended, but varies depending on the type of oil, degree of weathering, temperature, and thickness of the slick. As with all dispersants, timely application ensures the highest degree of success.

TOXICITY

Human

Toxicity tests have not been conducted on humans; however it is not considered a carcinogen by the International Agency for Research on Cancer, the National Toxicology Program, or the American Conference of Governmental Industrial Hygienists. First aid measures that should be taken if exposure occurs include flushing the eyes with water, wash skin with soap and water, and removing the victim to fresh air. For all contact including ingestion or inhalation the victim should seek medical attention. The human hazard characterization is high.

Ecological

Based on the recommended product application and the product's characteristics the potential environmental exposure is low. COREXIT 9527 component substances have a low potential to bioconcentrate.

EFFECTIVENESS

The dispersant effectiveness was conducted using the swirling flask test with South Louisiana (S/L) and Prudhoe Bay (P/B) crude oils. It was found to be 63.4% effective on S/L and 37.4% effective on P/B.

PHYSICAL PROPERTIES

1. Flash Point: 163°F
2. Pour Point: Less than -40°F
3. Viscosity: 160 cst at 32°F
4. Specific Gravity: 0.98 – 1.02
5. pH: 6.1
6. Chemical Name and Percentage by Weight of the Total Formulation: CONFIDENTIAL
7. Surface Active Agents: CONFIDENTIAL
8. Solvents: Water, Propylene Glycol, 2-Butoxyethanol
9. Additives: CONFIDENTIAL
10. Solubility: Complete

**MATERIAL SAFETY DATA SHEET****PRODUCT****COREXIT® 9500****EMERGENCY TELEPHONE NUMBER(S)****(800) 424-9300 (24 Hours) CHEMTREC****1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION**

PRODUCT NAME : COREXIT® 9500
APPLICATION : OIL SPILL DISPERSANT
COMPANY IDENTIFICATION : Naico Energy Services, L.P.
P.O. Box 87
Sugar Land, Texas
77487-0087
EMERGENCY TELEPHONE NUMBER(S) : (800) 424-9300 (24 Hours) CHEMTREC

NFPA 704M/HMIS RATING

HEALTH : 1 / 1 FLAMMABILITY : 1 / 1 INSTABILITY : 0 / 0 OTHER :
0 = Insignificant 1 = Slight 2 = Moderate 3 = High 4 = Extreme

2. COMPOSITION/INFORMATION ON INGREDIENTS

Our hazard evaluation has identified the following chemical substance(s) as hazardous. Consult Section 15 for the nature of the hazard(s).

Hazardous Substance(s)	CAS NO	% (w/w)
Distillates, petroleum, hydrotreated light	64742-47-8	10.0 - 30.0
Propylene Glycol	57-55-6	1.0 - 5.0
Organic sulfonic acid salt	Proprietary	10.0 - 30.0

3. HAZARDS IDENTIFICATION****EMERGENCY OVERVIEW******WARNING****Combustible.**

Keep away from heat. Keep away from sources of ignition - No smoking. Keep container tightly closed. Do not get in eyes, on skin, on clothing. Do not take internally. Avoid breathing vapor. Use with adequate ventilation. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of soap and water.

Wear suitable protective clothing.

Low Fire Hazard; liquids may burn upon heating to temperatures at or above the flash point. May evolve oxides of carbon (COx) under fire conditions. May evolve oxides of sulfur (SOx) under fire conditions.

PRIMARY ROUTES OF EXPOSURE :

Eye, Skin

HUMAN HEALTH HAZARDS - ACUTE :**EYE CONTACT :**

May cause irritation with prolonged contact.

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SKIN CONTACT :

May cause irritation with prolonged contact.

INGESTION :

Not a likely route of exposure. Can cause chemical pneumonia if aspirated into lungs following ingestion.

INHALATION :

Repeated or prolonged exposure may irritate the respiratory tract.

SYMPTOMS OF EXPOSURE :**Acute :**

A review of available data does not identify any symptoms from exposure not previously mentioned.

Chronic :

Frequent or prolonged contact with product may defat and dry the skin, leading to discomfort and dermatitis.

AGGRAVATION OF EXISTING CONDITIONS :

Skin contact may aggravate an existing dermatitis condition.

4. FIRST AID MEASURES**EYE CONTACT :**

Immediately flush with plenty of water for at least 15 minutes. If symptoms develop, seek medical advice.

SKIN CONTACT :

Immediately wash with plenty of soap and water. If symptoms develop, seek medical advice.

INGESTION :

Do not induce vomiting: contains petroleum distillates and/or aromatic solvents. If conscious, washout mouth and give water to drink. Get medical attention.

INHALATION :

Remove to fresh air, treat symptomatically. Get medical attention.

NOTE TO PHYSICIAN :

Based on the individual reactions of the patient, the physician's judgement should be used to control symptoms and clinical condition.

5. FIRE FIGHTING MEASURES

FLASH POINT : 181.4 °F / 83 °C (PMCC)

LOWER EXPLOSION LIMIT : Not flammable

UPPER EXPLOSION LIMIT : Not flammable



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EXTINGUISHING MEDIA :

Alcohol foam, Carbon dioxide, Foam, Dry powder, Other extinguishing agent suitable for Class B fires, For large fires, use water spray or fog, thoroughly drenching the burning material.

Water mist may be used to cool closed containers.

UNSUITABLE EXTINGUISHING MEDIA :

Do not use water unless flooding amounts are available.

FIRE AND EXPLOSION HAZARD :

Low Fire Hazard; liquids may burn upon heating to temperatures at or above the flash point. May evolve oxides of carbon (COx) under fire conditions. May evolve oxides of sulfur (SOx) under fire conditions.

SPECIAL PROTECTIVE EQUIPMENT FOR FIRE FIGHTING :

In case of fire, wear a full face positive-pressure self contained breathing apparatus and protective suit.

6. ACCIDENTAL RELEASE MEASURES

PERSONAL PRECAUTIONS :

Restrict access to area as appropriate until clean-up operations are complete. Stop or reduce any leaks if it is safe to do so. Ventilate spill area if possible. Do not touch spilled material. Remove sources of ignition. Have emergency equipment (for fires, spills, leaks, etc.) readily available. Use personal protective equipment recommended in Section 8 (Exposure Controls/Personal Protection). Notify appropriate government, occupational health and safety and environmental authorities.

METHODS FOR CLEANING UP :

SMALL SPILLS: Soak up spill with absorbent material. Place residues in a suitable, covered, properly labeled container. Wash affected area. **LARGE SPILLS:** Contain liquid using absorbent material, by digging trenches or by diking. Reclaim into recovery or salvage drums or tank truck for proper disposal. Clean contaminated surfaces with water or aqueous cleaning agents. Contact an approved waste hauler for disposal of contaminated recovered material. Dispose of material in compliance with regulations indicated in Section 13 (Disposal Considerations).

ENVIRONMENTAL PRECAUTIONS :

Do not contaminate surface water.

7. HANDLING AND STORAGE

HANDLING :

Use with adequate ventilation. Keep the containers closed when not in use. Do not take internally. Do not get in eyes, on skin, on clothing. Have emergency equipment (for fires, spills, leaks, etc.) readily available.

STORAGE CONDITIONS :

Store away from heat and sources of ignition. Store separately from oxidizers. Store the containers tightly closed.

SUITABLE CONSTRUCTION MATERIAL :

Compatibility with Plastic Materials can vary; we therefore recommend that compatibility is tested prior to use.

**MATERIAL SAFETY DATA SHEET****PRODUCT****COREXIT® 9500****EMERGENCY TELEPHONE NUMBER(S)****(800) 424-9300 (24 Hours) CHEMTREC****8. EXPOSURE CONTROLS/PERSONAL PROTECTION****OCCUPATIONAL EXPOSURE LIMITS :**

Exposure guidelines have not been established for this product. Available exposure limits for the substance(s) are shown below.

ACGIH/TLV :

Substance(s)

Oil Mist

TWA: 5 mg/m³STEL: 10 mg/m³

Propylene Glycol

OSHA/PEL :

Substance(s)

Oil Mist

TWA: 5 mg/m³STEL: 10 mg/m³

Propylene Glycol

AIHA/WEEL :

Substance(s)

ENGINEERING MEASURES :

General ventilation is recommended.

RESPIRATORY PROTECTION :

Where concentrations in air may exceed the limits given in this section, the use of a half face filter mask or air supplied breathing apparatus is recommended. A suitable filter material depends on the amount and type of chemicals being handled. Consider the use of filter type: Multi-contaminant cartridge, with a Particulate pre-filter. In event of emergency or planned entry into unknown concentrations a positive pressure, full-facepiece SCBA should be used. If respiratory protection is required, institute a complete respiratory protection program including selection, fit testing, training, maintenance and inspection.

HAND PROTECTION :

Nitrile gloves, PVC gloves

SKIN PROTECTION :

Wear standard protective clothing.

EYE PROTECTION :

Wear chemical splash goggles.

HYGIENE RECOMMENDATIONS :

Keep an eye wash fountain available. Keep a safety shower available. If clothing is contaminated, remove clothing and thoroughly wash the affected area. Launder contaminated clothing before reuse.

HUMAN EXPOSURE CHARACTERIZATION :

Based on our recommended product application and personal protective equipment, the potential human exposure is: Low

**MATERIAL SAFETY DATA SHEET**

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9. PHYSICAL AND CHEMICAL PROPERTIES

PHYSICAL STATE	Liquid
APPEARANCE	Clear Hazy Amber
ODOR	Hydrocarbon
SPECIFIC GRAVITY	0.95 @ 60 °F / 15.6 °C
DENSITY	7.91 lb/gal
SOLUBILITY IN WATER	Miscible
pH (100 %)	6.2
VISCOSITY	177 cps @ 32 °F / 0 °C 70 cps @ 60 °F / 15.6 °C @ 104 °F / 40 °C
VISCOSITY	@ 32 °F / 0 °C @ 60 °F / 15.6 °C 22.5 cst @ 104 °F / 40 °C
POUR POINT	< -71 °F / < -57 °C
BOILING POINT	296 °F / 147 °C
VAPOR PRESSURE	15.5 mm Hg @ 100 °F / 37.8 °C

Note: These physical properties are typical values for this product and are subject to change.

10. STABILITY AND REACTIVITY

STABILITY :

Stable under normal conditions.

HAZARDOUS POLYMERIZATION :

Hazardous polymerization will not occur.

CONDITIONS TO AVOID :

Heat

MATERIALS TO AVOID :

Contact with strong oxidizers (e.g. chlorine, peroxides, chromates, nitric acid, perchlorate, concentrated oxygen, permanganate) may generate heat, fires, explosions and/or toxic vapors.

HAZARDOUS DECOMPOSITION PRODUCTS :

Under fire conditions: Oxides of carbon, Oxides of sulfur

11. TOXICOLOGICAL INFORMATION

No toxicity studies have been conducted on this product.

SENSITIZATION :

This product is not expected to be a sensitizer.

**MATERIAL SAFETY DATA SHEET**

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CARCINOGENICITY :

None of the substances in this product are listed as carcinogens by the International Agency for Research on Cancer (IARC), the National Toxicology Program (NTP) or the American Conference of Governmental Industrial Hygienists (ACGIH).

HUMAN HAZARD CHARACTERIZATION :

Based on our hazard characterization, the potential human hazard is: Moderate

12. ECOLOGICAL INFORMATION**ECOTOXICOLOGICAL EFFECTS :**

The following results are for the product.

ACUTE INVERTEBRATE RESULTS :

Species	Exposure	LC50	EC50	Test Descriptor
Acartia tonsa	48 hrs	34 mg/l		Product
Artemia	48 hrs	20.7 mg/l		Product

MOBILITY :

The environmental fate was estimated using a level III fugacity model embedded in the EPI (estimation program interface) Suite TM, provided by the US EPA. The model assumes a steady state condition between the total input and output. The level III model does not require equilibrium between the defined media. The information provided is intended to give the user a general estimate of the environmental fate of this product under the defined conditions of the models. If released into the environment this material is expected to distribute to the air, water and soil/sediment in the approximate respective percentages;

Air	Water	Soil/Sediment
<5%	10 - 30%	50 - 70%

The portion in water is expected to float on the surface.

BIOACCUMULATION POTENTIAL

Component substances have a potential to bioconcentrate.

ENVIRONMENTAL HAZARD AND EXPOSURE CHARACTERIZATION

Based on our hazard characterization, the potential environmental hazard is: Low

Based on our recommended product application and the product's characteristics, the potential environmental exposure is: Low

If released into the environment, see CERCLA/SUPERFUND in Section 15.

13. DISPOSAL CONSIDERATIONS

If this product becomes a waste, it could meet the criteria of a hazardous waste as defined by the Resource Conservation and Recovery Act (RCRA) 40 CFR 261. Before disposal, it should be determined if the waste meets the criteria of a hazardous waste.

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Hazardous Waste: D018

Hazardous wastes must be transported by a licensed hazardous waste transporter and disposed of or treated in a properly licensed hazardous waste treatment, storage, disposal or recycling facility. Consult local, state, and federal regulations for specific requirements.

14. TRANSPORT INFORMATION

The information in this section is for reference only and should not take the place of a shipping paper (bill of lading) specific to an order. Please note that the proper Shipping Name / Hazard Class may vary by packaging, properties, and mode of transportation. Typical Proper Shipping Names for this product are as follows.

LAND TRANSPORT :

For Packages Less Than Or Equal To 119 Gallons:

Proper Shipping Name :

PRODUCT IS NOT REGULATED DURING TRANSPORTATION

For Packages Greater Than 119 Gallons:

Proper Shipping Name :

COMBUSTIBLE LIQUID, N.O.S.

Technical Name(s) :

PETROLEUM DISTILLATES

UN/ID No :

NA 1993

Hazard Class - Primary :

COMBUSTIBLE

Packing Group :

III

Flash Point :

83 °C / 181.4 °F**AIR TRANSPORT (ICAO/IATA) :**

Proper Shipping Name :

PRODUCT IS NOT REGULATED DURING TRANSPORTATION**MARINE TRANSPORT (IMDG/IMO) :**

Proper Shipping Name :

PRODUCT IS NOT REGULATED DURING TRANSPORTATION**15. REGULATORY INFORMATION****NATIONAL REGULATIONS, USA :****OSHA HAZARD COMMUNICATION RULE, 29 CFR 1910.1200 :**

Based on our hazard evaluation, the following substance(s) in this product is/are hazardous and the reason(s) is/are shown below.

Distillates, petroleum, hydrotreated light : Irritant

Propylene Glycol : Exposure Limit, Eye irritant.

Organic sulfonic acid salt : Irritant

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CERCLA/SUPERFUND, 40 CFR 117, 302 :
Notification of spills of this product is not required.

SARA/SUPERFUND AMENDMENTS AND REAUTHORIZATION ACT OF 1986 (TITLE III) - SECTIONS 302, 311, 312, AND 313 :

SECTION 302 - EXTREMELY HAZARDOUS SUBSTANCES (40 CFR 355) :
This product does not contain substances listed in Appendix A and B as an Extremely Hazardous Substance.

SECTIONS 311 AND 312 - MATERIAL SAFETY DATA SHEET REQUIREMENTS (40 CFR 370) :
Our hazard evaluation has found this product to be hazardous. The product should be reported under the following indicated EPA hazard categories:

- X Immediate (Acute) Health Hazard
- Delayed (Chronic) Health Hazard
- Fire Hazard
- Sudden Release of Pressure Hazard
- Reactive Hazard

Under SARA 311 and 312, the EPA has established threshold quantities for the reporting of hazardous chemicals. The current thresholds are: 500 pounds or the threshold planning quantity (TPQ), whichever is lower, for extremely hazardous substances and 10,000 pounds for all other hazardous chemicals.

SECTION 313 - LIST OF TOXIC CHEMICALS (40 CFR 372) :
This product does not contain substances on the List of Toxic Chemicals.

TOXIC SUBSTANCES CONTROL ACT (TSCA) :
The substances in this preparation are included on or exempted from the TSCA 8(b) Inventory (40 CFR 710)

FEDERAL WATER POLLUTION CONTROL ACT, CLEAN WATER ACT, 40 CFR 401.15 / formerly Sec. 307, 40 CFR 116.4 / formerly Sec. 311 :
None of the substances are specifically listed in the regulation.

CLEAN AIR ACT, Sec. 111 (40 CFR 60, Volatile Organic Compounds), Sec. 112 (40 CFR 61, Hazardous Air Pollutants), Sec. 602 (40 CFR 82, Class I and II Ozone Depleting Substances) :
None of the substances are specifically listed in the regulation.

Substance(s)	Citations
• Propylene Glycol	Sec. 111

CALIFORNIA PROPOSITION 65 :
This product does not contain substances which require warning under California Proposition 65.

MICHIGAN CRITICAL MATERIALS :
None of the substances are specifically listed in the regulation.



MATERIAL SAFETY DATA SHEET

PRODUCT

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STATE RIGHT TO KNOW LAWS :

The following substances are disclosed for compliance with State Right to Know Laws:

Propylene Glycol

57-55-6

NATIONAL REGULATIONS, CANADA :

WORKPLACE HAZARDOUS MATERIALS INFORMATION SYSTEM (WHMIS) :

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

WHMIS CLASSIFICATION :

Not considered a WHMIS controlled product.

CANADIAN ENVIRONMENTAL PROTECTION ACT (CEPA) :

The substances in this preparation are listed on the Domestic Substances List (DSL), are exempt, or have been reported in accordance with the New Substances Notification Regulations.

16. OTHER INFORMATION

Due to our commitment to Product Stewardship, we have evaluated the human and environmental hazards and exposures of this product. Based on our recommended use of this product, we have characterized the product's general risk. This information should provide assistance for your own risk management practices. We have evaluated our product's risk as follows:

* The human risk is: Low

* The environmental risk is: Low

Any use inconsistent with our recommendations may affect the risk characterization. Our sales representative will assist you to determine if your product application is consistent with our recommendations. Together we can implement an appropriate risk management process.

This product material safety data sheet provides health and safety information. The product is to be used in applications consistent with our product literature. Individuals handling this product should be informed of the recommended safety precautions and should have access to this information. For any other uses, exposures should be evaluated so that appropriate handling practices and training programs can be established to insure safe workplace operations. Please consult your local sales representative for any further information.

REFERENCES

Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices, American Conference of Governmental Industrial Hygienists, OH., (Ariel Insight# CD-ROM Version), Ariel Research Corp., Bethesda, MD.

Hazardous Substances Data Bank, National Library of Medicine, Bethesda, Maryland (TOMES CPS# CD-ROM Version), Micromedex, Inc., Englewood, CO.

**MATERIAL SAFETY DATA SHEET****PRODUCT****COREXIT® 9500****EMERGENCY TELEPHONE NUMBER(S)****(800) 424-9300 (24 Hours) CHEMTREC**

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Geneva: World Health Organization, International Agency for Research on Cancer.

Integrated Risk Information System, U.S. Environmental Protection Agency, Washington, D.C. (TOMES CPS# CD-ROM Version), Micromedex, Inc., Englewood, CO.

Annual Report on Carcinogens, National Toxicology Program, U.S. Department of Health and Human Services, Public Health Service.

Title 29 Code of Federal Regulations, Part 1910, Subpart Z, Toxic and Hazardous Substances, Occupational Safety and Health Administration (OSHA), (Ariel Insight# CD-ROM Version), Ariel Research Corp., Bethesda, MD.

Registry of Toxic Effects of Chemical Substances, National Institute for Occupational Safety and Health, Cincinnati, OH, (TOMES CPS# CD-ROM Version), Micromedex, Inc., Englewood, CO.

Ariel Insight# (An integrated guide to industrial chemicals covered under major regulatory and advisory programs), North American Module, Western European Module, Chemical Inventories Module and the Generics Module (Ariel Insight# CD-ROM Version), Ariel Research Corp., Bethesda, MD.

The Teratogen Information System, University of Washington, Seattle, WA (TOMES CPS# CD-ROM Version), Micromedex, Inc., Englewood, CO.

Prepared By : Product Safety Department
Date issued : 06/14/2005
Version Number : 1.6

May 20, 2010

Rear Admiral Mary Landry
Commander, Eighth Coast Guard District
Hale Boggs Federal Building
500 Poydras Street
New Orleans, LA 70130

Samuel Coleman, P.E.
Director, Superfund Division
U.S. EPA Region 6
Dallas, TX 75202

Re: May 19, 2010 Addendum 2 to Dispersant Monitoring and Assessment Directive ("Addendum 2")

Dear Admiral Landry and Mr. Coleman:

This letter is the response to the directive in Addendum 2 for BP Exploration & Production Inc. ("BP") to identify within 24 hours of issuance of Addendum 2 one or more approved dispersant products from the National Contingency Plan Product Schedule that are "available in sufficient quantities, are as effective at dispersing the oil plume, and have a toxicity value less than or equal to 23.00 ppm LC50 toxicity value for *Menidia* or 18.00 ppm LC50 for *Mysidopsis*, as indicated on the NCP Product Schedule".

BP's response below considers the criteria set forth in the directive in the following order (1) dispersants with a toxicity value greater than or equal to 32.00 ppm LC50 toxicity value for *Menidia* or 18.00 ppm LC50 for *Mysidopsis*, as indicated on the NCP Product Schedule, (2) the availability based on existing stockpiles, the estimated time to begin aerial and subsurface application, and time for manufacturing, shipping and warehousing, and (3) as effective as Corexit EC9500A at dispersing the oil plume. As discussed below, given the above criteria, BP continues to believe that Corexit EC9500A is the best alternative.

(1) Toxicity Value.

Only five products on the NCP Product Schedule meet the criteria in the May 19th directive. These are: Sea Brat #4, Nokomis 3-F4 and Nokomis 3-AA, Mare Clean 200, and Neos AB3000.

EPA has used acute toxicity criteria to evaluate dispersants that will be applied to oil floating on the water surface. When evaluating the same materials for subsea use, additional criteria may be relevant. We have attached a summary of the criteria that BP is using to evaluate dispersant options, and comparison tables that evaluate each dispersant by such criteria, based on information currently available to us.

One relevant criterion, given the amount of dispersant that is required at this site and the proposed application near the ocean floor, is the potential long term effect and persistence of the chemicals in each dispersant.

In this regard, Sea Brat #4 contains a small amount of a chemical that may degrade to a nonylphenol (NP). The class of NP chemicals have been identified by various government agencies as potential endocrine disruptors, and as chemicals that may persist in the environment for a period of years. The manufacturer has not had the opportunity to evaluate this product for those potential effects, and BP has not had the opportunity to conduct independent tests to evaluate this issue either. BP learned of this issue after it applied for permission to use Sea Brat #4 at the incident site.

With this additional information in hand, we believe it would be prudent to evaluate the potential NP issue more carefully before EPA or the FOSC require Sea Brat to be used at the incident site, and in particular, before it is applied underwater near the ocean floor.

It would also be prudent to obtain the chemical formulas for the other dispersants that meet the acute toxicity criteria in the May 19th directive, and evaluate them for their potential to degrade to NP, or any other chemical that has been identified as a potential endocrine disruptor. BP has not been able to obtain this information in the 24 hour time frame provided in the directive.

COREXIT does not contain chemicals that degrade to NP. The manufacturer indicates that COREXIT reaches its maximum biodegradability within 28 days of application, and that it does not persist in the environment. These qualities make COREXIT a better choice for subsea application, based on the information currently available. COREXIT appears to have fewer long term effects than the other dispersants evaluated.

(2) Availability.

BP has an inventory of 246,380 gallons of COREXIT that are available for immediate use, and the manufacturer is able to produce an additional 68,000 gallons/day, which is sufficient to meet all anticipated dispersant needs at this site.

BP also has an inventory of 100,000 gallons of Sea Brat #4 available for immediate use. The manufacturer is able to produce an additional [] gallons/day, which would be sufficient to meet all anticipated surface application needs, but may not be sufficient to meet both surface and subsurface application needs combined.

BP does not have a stockpile of the other dispersants that meet the criteria in the May 19th Directive, and the manufacturers tell us that they cannot produce the requested volume for 10 to 14 days or more.

Attached to this letter is a table that describes the availability and production capability for each dispersant option (See "Dispersant Supply Profile.")

(3) Effectiveness.

COREXIT was 55% to 63% effective in dispersing samples of South Louisiana Crude Oil. Sea Brat #4 was 61% effective in dispersing samples of the same material. The products are expected to have similar levels of effectiveness in the field.

Attached to this letter is a table that shows the expected effectiveness ratings for the four other dispersants that meet the acute toxicity criteria in Addendum 2. The Nokomis products are slightly more effective (64-65%), while Mare Clean and Neos AB3000 are reported to be substantially more effective at dispersing oil (84% and 90%).

(4) Conclusion.

In the midst of an oil spill response, one of the most important criteria is whether the dispersant in question can be obtained in sufficient volumes to meet immediate needs. Dispersants must be applied to the spill shortly after release to be effective. As oil weathers in the environment, it becomes increasingly difficult to disperse with any of the listed products.

COREXIT was the only dispersant that was available immediately, in sufficiently large quantities, to be useful at the time of the spill. Subsequent efforts have identified Sea Brat #4 as a possible alternative that is equally effective at dispersing oil, but has fewer acute toxicity effects. In the short

time provided to us, BP and the manufacturer of Sea Brat #4 have not had the opportunity to evaluate other potentially significant criteria, including the risk that a small fraction of Sea Brat #4 may degrade to NP, and/or may persist in the environment.

None of the other dispersants that meet the acute toxicity and effectiveness criteria in Addendum 2 are available in sufficient quantities at this time. In addition, before supporting a decision to switch to those dispersants, it would be important to review the formula for each alternative, and evaluate it for additional risks, such as persistence in the environment. BP has not been able to do this in the time provided.

Based on the information that is available today, BP continues to believe that COREXIT was the best and most appropriate choice at the time when the incident occurred, and that COREXIT remains the best option for subsea application.

Before the Coast Guard and EPA issue further directives requiring a change in dispersant products or monitoring, we would appreciate the opportunity to meet with you to discuss the options and their efficacy and potential impacts, in view of the circumstances at the spill site, and the proposed methods of usage.

After you have the opportunity to review the attached information, please let me know the earliest time when you might be available to meet with our team to discuss these issues.

Sincerely,

Douglas J. Suttles

I. INTRODUCTION

This attachment contains detailed technical information in response to the directive addendum from the U.S. Coast Guard (USCG) and the Environmental Protection Agency (EPA), directing BP to identify “one or more approved dispersant products from the National Contingency Plan Schedule that are available in sufficient quantities, are as effective at dispersing the oil plume, and have a toxicity value [greater]¹ than or equal to 23.00 ppm LC50 toxicity value for *Menidia* or 18.00 ppm LC50 for *Mysidopsis*.” See Dispersant Monitoring and Assessment Directive - Addendum, dated May 19, 2010 (“May 19th Directive”).

To respond to the short deadline contained in the May 19th Directive, the information that we can provide is necessarily limited to the information that was in hand or could be obtained on 24 hours notice.

II. BACKGROUND

By way of background, and to provide some context, we begin by briefly describing why COREXIT was selected and approved for use by the EPA and the USCG. COREXIT is on the list of dispersants that are pre-approved for surface application to oil. It is one of the most commonly used dispersants, and has been used before in the Gulf of Mexico. Most important is that it was possible to quickly obtain a large enough supply of COREXIT to meet the anticipated needs at this site, by purchasing it from the manufacturer and by borrowing it from other companies. No other dispersant was available in the required amounts at the time of the oil spill.

III. POTENTIAL ALTERNATIVE DISPERSANTS

BP has identified the following dispersant products as potential alternatives to the COREXIT products approved for use:

1. Dispersit SPC 1000;
2. JD 2000;
3. Mare Clean 200;
4. Neos AB3000; and
5. Nokomis 3-AA;
6. Nokomis 3-F4
7. SAF-RON Gold;

¹ The directive says “less than or equal to,” but BP presumes that the intended expression was “greater than or equal to,” since lower toxicity values indicate higher toxicity.

8. Sea Brat #4;

The Mare Clean 200, Neos AB3000, Nokomis 3-AA, Nokomis 3-F4 and Sea Brat #4 all have LC50 values greater than or equal to either the *Menidia* or *Mysidopsis* criteria, as required by the May 19th Directive.

IV. EVALUATION CRITERIA

In the table in section __ below, BP provides nine categories of information to assist the USCG and EPA in choosing alternative dispersants for use in the Spill Response. These categories are the following:

A. NCP Product Schedule Listing

Pursuant to Subpart J of the National Oil and Hazardous Substances Pollution Contingency Plan, no dispersant may be used in the United States if it is not listed on the National Contingency Plan Product Schedule. Accordingly, the only dispersant products being considered for possible use in the spill response are among those currently listed on the NCP National Product Schedule.

B. Effectiveness in Laboratory Trials

Each dispersant must be tested for effectiveness before it is listed in the Product Schedule. In addition, pursuant to EPA and U.S. Coast Guard approval, samples of Dispersit SPC 1000, JD-2000, Nokomis 3-AA, SAF-RON Gold, and Sea Brat #4 were tested in the laboratory for their effectiveness in dispersing oil using both the swirling task method (EPA-approved method) and a modified EXDET (Exxon Dispersant Effectiveness Test).² The test oil used was a surrogate from the nearby Thunder Hawk rig since fresh crude oil from the MC 252 was unavailable at the time.

C. Effectiveness in Field Trials

Actual field trials can provide a more accurate assessment of the potential performance of dispersants than laboratory trials. Field trials on MC 252 oil in various stages of weathering have been completed for Nalco EC 9500A.

D. Acute Toxicity

Each dispersant must be tested for acute toxicity before it is listed in the Product Schedule. In addition, we have reviewed and will continue to review information available from

² The EXDET test measures relative dispersant effectiveness, allows comparisons among small-scale laboratory tests, and assists with comparisons to field trials (Becker, K.W., L.G. Coker, and M.A. Walsh. 1991. "A method for evaluating oil spill dispersants, Exxon Dispersant Effectiveness Test (EXDET)" in *Oceans '91 Proceedings*, Oceanic Engineering Society of IEEE, New York, NY. pp. 1486-1490).

material data safety sheets (MSDS), toxicity information available from the National Product Schedule, information provided by manufacturers and information available in scientific literature.

E. Persistence, Bioaccumulation and Chronic Effects and Endocrine Disruption

BP is reviewing available information about the persistence, bioaccumulation, chronic effects, endocrine disruption and other impacts of each dispersant to determine which dispersants will have the fewest impacts overall, and not just the best performance on the tests for the Product Schedule. There may be only limited data on long-term impacts for many of the dispersants as formulated, however. In addition, there may be only limited information on the constituents of the dispersants, since the dispersants typically contain proprietary substances whose identities are not publicly available. For those dispersants where constituents and/or data are publicly available, BP will identify and catalogue long-term impacts. For those where constituents are not publicly available, BP will endeavor to obtain confidential information about the constituents so that we may identify long-term impacts and review them with the EPA in a confidential manner.

*** CONFIDENTIAL INFORMATION BELOW***

[

]

NP is a potential endocrine disrupter that has been mentioned by the U.S. EPA's Endocrine Disruption Screening Program, and the EPA has developed final marine acute and chronic water quality criteria developed for NP. NP also has been reviewed under the U.S. EPA's Great Lakes Binational Strategy, is on the OSPAR list of hazardous constituents for discharge into the sea, and is a priority hazardous pollutant under EU Water Directive.

This regulatory attention notwithstanding, NP is still widely used in consumer and agricultural products, and is regularly detected in wastewater treatment plant effluent. For example, Kolpin et al (2002) reported on a 1999-2000 survey of 85 sample sites across the U.S. (freshwater) that NP concentrations averaged 0.8 ug/L.

If a dispersant with NPE levels comparable to those of [] is used on the spill, the acute criteria may be temporarily exceeded shortly after application, depending on the thickness of the oil slick and the amount of dispersant applied. Exceedances of the chronic criteria appear unlikely, but could occur if [] is applied in the same area over a period of several days. Whether or not the acute criterion will be exceeded largely depends on the interval between applications.

For NP at or near the surface, photochemical transformation can be a significant route of abiotic degradation, according to a literature review conducted by Melcer et. al. (2007). Under simulated summer sunlight conditions in the surface layer of natural waters, NP's half-life has been estimated as less than a day.

For NP in dark, anoxic environments such as deep water sediments, however, available information suggests much slower degradation.

*** CONFIDENTIAL INFORMATION ABOVE***

F. Whether Potential Alternatives Have Been Prohibited Outside the United States

As part of our evaluation of the COREXIT products approved for use, BP has reviewed available information concerning their use outside the United States.³ BP has conducted similar research for the 8 potential alternatives products. To date, we are not aware that any have been prohibited by any foreign regulators.

G. Behavior in the Environment

The behavior of dispersants in the environment may affect both its effectiveness and its long term impacts. One factor determining the behavior of dispersants after application is the tendency of a dispersant to rise or sink in the water column which, in turn, depends on whether the dispersants contain significant quantities of petroleum-based solvents that are less dense than water. Two other factors are the biodegradation of the dispersant and its tendency to bioaccumulate and bioconcentrate.

H. Quantities Currently Available and Reliability of Supply

An important consideration in identifying and selecting possible alternative dispersants is the commercial availability of those products in quantities sufficient to meet current and anticipated needs. Approximately 75,000 gallons of dispersant is used each day for surface

³ We have learned that COREXIT 9527 and COREXIT 9500 were removed from the list of approved dispersants in the UK. Our understanding is that these two products were removed due to a new test added by the UK regulators. The test, known as the "rocky shores test," is designed to evaluate the toxicity of the dispersants when sprayed in the tidal zone, and the mortality of limpets exposed to the dispersant. The test was added because of concerns that dispersants may cause more significant ecological impacts on rocky shores than they do on sandy or pebble beaches (primarily seaweed overgrowth due to increased mortality in the harvester species). The UK regulators continue to allow the use of existing stockpiles of these COREXIT products away from rocky shorelines, with approval. We have not been informed by the On Scene Coordinator that the "rocky shores test" is applicable to the conditions in the Gulf, as most tidal areas near the release are not rocky, and again US EPA and Coast Guard have approved both products for use in this response.

and subsea application. Going forward, an estimated 50,000 gallons per day will be needed for continued aerial spraying. It is also important to consider the extent to which a manufacturer can reliably produce and deliver sufficient quantities of quality-grade product to the field. Therefore, we have and will continue to evaluate any potential supply chain problems (e.g., interruptions in the manufacturer's ability to obtain raw materials needed to make the product), quality control issues (e.g., production of significant volumes off-specification product that is ineffective in dispersing oil and could not be used) and delivery problems (e.g., inability to arrange timely transport of the product to the field).

V. Available Data on the Potential Alternatives

In the following table, BP has compiled the available information relevant to the dispersants and criteria described above.

Evaluation Criteria for Selected Dispersants									
Evaluation Criteria	Comment	Corexit® EC9500A	Corexit® EC9527A	JD-2000	Dispersit SPC 1000™	Nokomis 3-F4	Sea Breat #4	Saf-Ron Gold	
A. NCP Product Schedule		Yes	Yes	Yes	Yes	Yes	Yes	Yes	
B. Effectiveness (EPA Swirl Test)	% Effective (Pruithoe Bay crude)	45.3	37.4	60.4	40	63.20	53.6	84.80	
	% Effective (South Louisiana crude)	54.7	63.4	77.8	100	63.70	60.7	53.80,	
	% Effective (Average)	50.0	50.4	69.1	73	64.50	57.1	69.30	
C. Effectiveness (Gulf Field Test)	Based on field test protocols developed by the Dispersant Operation Group				Not yet tested	Not yet tested	May 8 field test indicated oil dispersed with formation of droplets with a likely median diameter <50 microns	Not yet tested	
D.1 Acute Toxicity Data (NCP Schedule)	<i>Mysidopsis bahia</i> (shrimp) 48hr LC50 (mg/L)	32.23	24.14	90.50	16.6	20.16	14.0	63.00	
	<i>Menidia beryllina</i> (inland silverside fish) 96hr LC50 (mg/L)	25.20	14.57	407.00	3.5	34.2	30.0	29.43	
	<i>Acartia tonsa</i> marine copepod 48hr LC50 (mg/L)	34	--	--	--	--	--	--	
D.2 Additional Acute Toxicity Data (from MSDS)	<i>Ariemina</i> (shrimp) 48hr LC50 (mg/L)	20.7	--	--	--	--	--	--	
	<i>Psetta maxima</i> (Turbot flatfish) 96hr LC50 (mg/L)	--	50	--	--	--	--	--	

Evaluation Criteria for Selected Dispersants									
Evaluation Criteria	Comment	Corexit® EC9500A	Corctix® EC9527A	JD-2000	Dispersif SPC 1000™	Nokomis 3-F4	Sea Brat #4	SaI-Ron Gold	
E. Persistence, Bioaccumulation and Chronic Effects and Endocrine Disruptors: Constituents	Based on Information Provided by Manufacturer	Proprietary Mixture	Proprietary Mixture	Proprietary Mixture	Proprietary Mixture	Formulations may contain nonylphenol polyethylenecarboxylates (NPE), which biodegrade to nonylphenol, a potential endocrine disruptor. NPE use restricted in EU, under review in U.S.	Proprietary Mixture	Proprietary Mixture	
G.1. Behavior in the Environment: Solvent	Based on Information Provided by Manufacturer	Petroleum based solvent with propylene glycol	2- butoxyethanol and propylene glycol	Proprietary mixture, insufficient information	Water based containing emulsifiers, dispersants, and water dilutable coupling solvent	Water and propylene glycol	Water and propylene glycol	Proprietary mixture, insufficient information	

Evaluation Criteria for Selected Dispersants

Evaluation Criteria	Comment	Corexit® EC9500A	Corexit® EC9527A	JD-2000	Dispersit SPC 1000™	Nokomis 3-F4	Sea Brat #4	Saf-Ron Gold
<p>G.2. Behavior in the Environment: Biodegradation</p>	<p>Based on Information Provided by Manufacturer</p>	<p>Manufacturer describes as biodegradable, majority of components expected to readily biodegrade</p>	<p>Manufacturer describes as biodegradable, majority of components expected to readily biodegrade</p>	<p>Proprietary mixture, insufficient information</p>	<p>Manufacturer describes as "completely biodegradable surfactants" - Proprietary Mixture Currently Insufficient Information to Assess Composition</p>	<p>Nonylphenol, degradation product of NPE, potentially resistant to biodegradation during subsurface application - Proprietary Mixture Currently Insufficient Information to Assess</p>	<p>MSDS describes product as highly biodegradable</p>	<p>Proprietary mixture, insufficient information</p>
<p>G.3. Behavior in the Environment: Potential for Bioaccumulation</p>	<p>Based on Information Provided by Manufacturer</p>	<p>Manufacturer reports component substances have a potential to bioaccumulate</p>	<p>Manufacturer reports component substances have a low potential to bioaccumulate</p>	<p>Proprietary mixture, insufficient information</p>	<p>Proprietary mixture, insufficient information</p>	<p>Proprietary mixture, insufficient information</p>	<p>Proprietary mixture, insufficient information</p>	<p>Proprietary mixture, insufficient information</p>
<p>H. Quantities Currently Available and Reliability of Supply</p>		<p>BP to provide</p>	<p>BP to provide</p>	<p>BP to provide</p>	<p>Anticipates increasing to 20,000 gallons per day, and possibly later to 60,000 gallons per day</p>	<p>BP to provide</p>	<p>BP to provide</p>	<p>BP to provide</p>

VI. Conclusions

As discussed above, there are many considerations that are relevant to selecting dispersants for use.

*** CONFIDENTIAL INFORMATION BELOW***

In addition, there may be significant concerns with certain of the constituents of the dispersants that we cannot yet evaluate because we lack the proprietary information to do so. We currently have such information only for Sea Brat #4, Corexit EC 9500A, Corexit EC 9527A, and SAF-RON Gold. Of these four, the two Corexits appear to have no constituents that raise issues over and above any that might be evident from the acute toxicity tests. [

]

The MSDS and patent information that are available for Disperit suggest that it does not contain NP or a chemical that would degrade to NP. However, this needs to be confirmed by a review of the current formula, which the manufacturer has not supplied to us.

*** CONFIDENTIAL INFORMATION ABOVE***

Comparative Toxicity of Eight Oil Dispersant Products on Two Gulf of Mexico Aquatic Test Species

**U.S. Environmental Protection Agency
Office of Research and Development**

U.S.EPA/ORD Contributors

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This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

1. Introduction

Large quantities of Louisiana sweet crude oil have been released into the Gulf of Mexico since the explosion of the Deepwater Horizon oil exploration platform on April 20, 2010. As part of the integrated response effort to mitigate the impact of the oil in the environment, the decision was made to use dispersants listed on the U.S. Environmental Protection Agency's (EPA) National Contingency Plan (NCP) Product Schedule (EPA 2010a). Dispersants are being applied offshore on the surface as well as underwater at the source of the leak. The EPA conducted independent studies to assess the relative acute toxicity of eight dispersants on the NCP Product Schedule.

This report summarizes results of the first phase of testing obtained from acute toxicity tests conducted with eight oil dispersants using two Gulf of Mexico aquatic species: (1) the mysid shrimp, *Americamysis bahia*, an aquatic invertebrate, and (2) the inland silverside, *Menidia beryllina*, a small estuarine fish. These species are standard test organisms used in a variety of EPA toxicity test methods. The eight dispersants tested were Corexit 9500A, Dispersit SPC1000, JD-2000, Nokomis 3-AA, Nokomis 3-F4, Saf-Ron Gold, Sea Brat #4 and Z1-400. The tests were conducted using an established contract testing laboratory and in compliance with the Good Laboratory Practice regulations as provided in EPA 40CFR160 (USEPA, 40CFR Part 160). The approach described herein utilized consistent test methodologies within a single laboratory which provided a means to assess acute toxicity estimates across dispersants and independently evaluate the NCP Product schedule toxicity information. The next phases of this study will examine the acute toxicity of Louisiana sweet crude oil and dispersant-sweet crude oil mixtures on mysids and *Menidia* – the results will be reported separately at a later date.

2. Test Methods

The acute toxicity test methods followed, with slight modification, the requirements specified in U.S. Environmental Protection Agency's 62 FR 15576, Appendix C of Part 300 – *Swirling Flask Dispersant Effectiveness Test, Revised Standard Dispersant Toxicity Test and Bioremediation Agent Effectiveness Test* (USEPA, 1997) and the EPA Test Method 821-R-02-012, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (USEPA, 2002). Specific modifications are shown in Appendix A.

The exposure concentration range for each dispersant was chosen to bracket the estimated median lethal concentration (LC50) values reported in the NCP Product Schedule. The LC50 is defined as the concentration of a substance causing mortality in 50% of test organisms for a specified time interval, in this case, 48-hours for the mysid test and 96-hours for the silverside test. The commercially available statistical software package, CETIS[®] was used for the calculation of LC50 values using an automated decision tree adapted from EPA for selection of the appropriate statistical method (CETIS, 2009; USEPA, 1994). Point estimate procedures used to calculate LC50 values included linear regression methods, the non-parametric Spearman-Kärber method and the binomial method. A qualitative comparison was made between LC50 values for the eight dispersants tested as well as with those available in the NCP Product Schedule. Note that the reproducibility of static acute tests among laboratories using the same species/toxicant combination has been reported to generally fall within a factor of 3.5 among laboratories when using nominal concentrations (unmeasured treatment concentrations) for both freshwater and marine species (USEPA, 1981). Given the use of whole organisms in these tests, some variation in response attributable to differences in parameters such as culture and acclimation conditions, stock populations or variable water quality is expected and acceptable.

3. Results - Mysid Toxicity Tests

3.1 Mysid Testing Schedule

Following the first round of eight acute toxicity tests, dispersant LC50s were greater than the highest concentration tested for four of the eight dispersants. Definitive acute toxicity tests were repeated using higher test concentrations for JD-2000, Saf-Ron Gold, Sea Brat #4 and ZI-400.

3.2 Mysid Test Acceptability

Control performance (without dispersant) met all criteria for an acceptable exposure in each test ($\geq 90\%$ survival). All water quality parameters were within ranges specified in the protocol with the exception of dissolved oxygen for the high test concentration (56 ppm) in the Nokomis 3-AA exposure at 24 hours, which was measured at 56% of saturation. As dissolved oxygen levels were $>60\%$ at other time points in the test and the toxicity was clearly dose

related, the departure observed in the 56 ppm concentration at 24 hours was not considered to have had a negative impact on the exposure with Nokomis 3-AA.

3.3 Mysid Toxicity Results

In the first series of acute toxicity tests, LC50 values and 95% confidence intervals were successfully determined for Corexit 9500A, Dispersit SPC 1000, Nokomis 3-AA and Nokomis 3-F4 and in the second series of acute tests, LC50s were calculated for JD-2000, Saf-Ron Gold, Sea Brat #4 and ZI400. Test results are summarized in Table 1.

The LC50 values for dispersant acute tests with mysids ranged from 12 ppm for Dispersit SPC1000 to 788 ppm for JD-200 (Table 1). EPA uses a five-step scale of toxicity categories to classify pesticides based on their acute toxicity to aquatic organisms: LC50 values of >100 ppm are considered practically nontoxic; >10 to 100 ppm as slightly toxic; > 1 to 10 ppm as moderately toxic; LC50s of 0.1 to 1 ppm as highly toxic and LC50s < 0.1 ppm as very highly toxic (USEPA, 2010b). Using this toxicity classification, Corexit 9500A, Dispersit SPC1000, Nokomis-3AA, Nokomis 3-F4, Sea Brat #4 and ZI-400 would be classified as slightly toxic whereas JD-2000 and Saf-Ron Gold would be classified as practically non-toxic to mysids (Table 1).

Based on comparison of LC50 values and 95% confidence intervals across the eight dispersants tested in the present study, the rank order toxicity (most to least toxic) of the dispersants to mysids was: (1) Dispersit SPC1000, (2) Nokomis 3-AA, (3) Nokomis 3-F4, Corexit 9500A, (4) ZI-400, Sea Brat #4, (5) Saf-Ron Gold, and (6) JD-2000.

Factor ratios were used to compare LC50s derived for the same species/dispersant combination from different laboratories. The factor ratios between LC50 values determined in this study and NCP reported LC50 values were calculated as a ratio by dividing the higher of the two LC50 values by the lower LC50 value for each of the eight dispersants, respectively (Table 1). As an example, using information from Table 1, the factor ratio for Corexit 9500A was determined as $42/32.2 = 1.3$. The factor ratios calculated for Corexit 9500A, Dispersit SPC1000, Nokomis 3-AA, Nokomis 3-F4, Saf-Ron Gold and ZI-400 were less than or equal to 2.6 which

was considered within normal inter-laboratory variability (USEPA, 1981). Results for JD-2000 and Sea Brat #4 showed lower toxicities (i.e., higher LC50s) with factor ratios of 8.7 and 4.6, respectively, compared to their reported NCP LC50 values.

3.4 Mysid Reference Toxicant Test

A 48-hr acute toxicity test was conducted with the standard reference toxicant, sodium dodecyl sulfate (SDS), to evaluate the relative sensitivity of the mysids used in the series of dispersant toxicity tests. The mysids tested with SDS were from the same population and age range used for dispersant testing. The 48-hr LC50 and 95% confidence interval calculated for SDS was 23 ppm [19-26 ppm] which was consistent with the reported NCP LC50 values for SDS.

4. Results - *Menidia* Toxicity Tests

4.1 Menidia Testing Schedule

Following the first round of acute toxicity tests, dispersant LC50s were determined to be greater than the highest concentration tested for two of the eight dispersants. Definitive acute toxicity tests were repeated using higher test concentrations for Corexit 9500A and JD-2000.

4.2 Menidia Test Acceptability

Control performance met all criteria for an acceptable exposure in each of the eight dispersant tests conducted ($\geq 90\%$). All water quality parameters were within ranges specified in the test protocol for *Menidia beryllina*.

4.3 Menidia Toxicity Results

In the first series of acute tests, LC50 values and 95% confidence intervals were successfully determined for Dispersit SPC 1000, Nokomis 3-AA, Nokomis 3-F4, Saf-Ron Gold, Sea Brat #4 and ZI-400. In the second series of repeat acute tests, an LC50 was calculated for Corexit 9500A but not for the dispersant JD-2000. These data are summarized in Table 2. In the case of JD-2000, 20% mortality was observed in the highest concentration tested of 5,600 ppm, followed by no mortality observed in the next two highest exposure concentrations which indicated an LC50 > 5,500 ppm. At the highest concentration, solid material was observed at the

bottom of the replicate test vessels suggesting saturation of the dispersant may have been achieved.

The LC50 values for dispersant acute toxicity tests with *Menidia* ranged from 2.9 ppm for Dispersit SPC1000 to 130 ppm for Corexit 9500A; the LC50 for JD 2000 exceeded the highest test concentration of 5,600 ppm. Using the EPA toxicity classification, Dispersit SPC1000 would be considered moderately toxic whereas Nokomis-3AA, Nokomis 3-F4, Saf-Ron Gold, Sea Brat #4 and ZI-400 would be classified as slightly toxic, and Corexit 9500A and JD-2000 as practically non-toxic to inland silversides.

Based on comparison of LC50 values and 95% confidence intervals, the rank order toxicity (most to least toxic) of the dispersants to *Menidia* were: (1) Disersit SPC1000, (2) Nokomis 3-F4, Nokomis 3-AA, ZI-400, (3) Saf-Ron Gold, (4) Sea Brat #4, (5) Corexit 9500A, and (6) JD-2000.

The factor ratios calculated for Dispersit SPC1000, Nokomis 3-AA, Nokomis 3-F4, Saf-Ron Gold, Sea Brat #4 and ZI-400 were less than or equal to 1.83 which was considered within normal inter-laboratory variability. The factor ratios of 5.2 and 13.8 for Corexit 9500A and JD-2000 indicate that the LC50 values reported for Corexit 9500A and JD-200 in the NCP Product Schedules would be considered different (i.e., lower) from the LC50 values determined in the present study.

Possible explanations for the 13.8 fold difference between the reported NCP LC50 for JD-2000 and the highest exposure concentration tested in the present study may be attributable to batch-to-batch variability in the manufacturing process, instability of the stored product over time, or a change in the product formulation.

4.4 *Menidia* Reference Toxicant Test

A 96-hr acute toxicity test was conducted with the reference toxicant SDS to evaluate the relative sensitivity of the *Menidia* used in the series of dispersant toxicity tests. The *Menidia* tested with SDS were from the same population and age range used for dispersant testing. The

96-hr LC50 and 95% confidence interval calculated for SDS was 9.5 ppm [8.7-10 ppm] which was consistent with the reported NCP LC50 values for SDS. It should be noted that during the last 24 hours of the test, the temperature dropped to 22°C, which was 2 degrees below the acceptable criteria and thus invalidated the test. However, there was no difference in mortality counts between the 72-hour and the 96-hour observations suggesting the temperature change had no negative impact on the test or the final calculated LC50.

5.0 Conclusions

The present study provided an independent, quantitative assessment of acute toxicities of eight dispersants to two aquatic species inhabiting Gulf of Mexico waters. Toxicity was determined as the LC50 derived from standard short term acute tests using standard test species, specifically the Gulf mysid, *Americamysis bahia*, and the inland silverside, *Menidia beryllina*. In general, the toxicity values (i.e., LC50s) for mysids ranged over nearly two orders of magnitude and for *Menidia* over three orders of magnitude. Given the expected range of inter-laboratory variability, the results of the present study were consistent with test results reported in the NCP Product Schedule, with the exception of two dispersants for each test species which yielded higher LC50s (i.e., lower toxicity) than reported in the NCP. The rank order toxicity of the eight dispersants was generally similar to the information provided in the NCP Product Schedule. For both test species, Dispersit SPC1000 was the most toxic and JD-2000 the least toxic. The other six dispersants varied in relative toxicity to mysids and *Menidia*, with LC50 values ranging from 20 to 130 ppm. Overall, the dispersants were classified as being slightly toxic to practically non-toxic to both test species, with the exception that Dispersit SPC1000 would be considered moderately toxic to *Menidia*. Corexit 9500A, the dispersant currently applied offshore at the surface and underwater, falls into the slightly toxic category for mysids and the practically non toxic category for *Menidia*.

Short-term acute toxicity tests using consistent methodologies and test organisms provide important and fundamental information on oil spill dispersants and other toxicants. The next phase of testing will examine the acute toxicity of Louisiana sweet crude oil and dispersant-sweet crude oil mixtures on mysids and *Menidia*. The comparative toxicity analysis of dispersants, sweet crude oil and dispersant-sweet crude oil mixtures on standard aquatic test

species will provide improved understanding of potential toxicological effects associated with this oil spill.

6.0 References

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Table 1. Results of mysid 48-hr static acute toxicity tests with eight dispersants. LC50 values (ppm), 95% confidence intervals [in brackets] and the toxicity classification of dispersant LC50s derived in the present study. NCP Product Schedule listing of dispersant LC50s and 95% confidence intervals [in brackets] shown in right column for comparison.

Dispersant	This Study LC50 (ppm) [95% CI]	Toxicity Category¹	NCP Product Schedule LC50 (ppm) [95% CI]^d
Dispersit SPC 1000	12 [10-14] ^a	Slightly Toxic	16.6 [14.1-19.6]
Nokomis 3-AA	30 [27-34] ^b	Slightly Toxic	20.2 [17.4-22.8]
Corexit 9500A	42 [38-47] ^c	Slightly Toxic	32.2 [26.5-39.2]
Nokomis 3-F4	42 [38-47] ^c	Slightly Toxic	32.2 [28.4-36.5]
ZI -400	55 [50-61] ^b	Slightly Toxic	21.0 [17.9-24.5]
Sea Brat #4	65 [57-74] ^a	Slightly Toxic	14.0 [±10.4]
Saf-Ron Gold	118 [104-133] ^b	Practically Non-Toxic	63.0 ^e [52.9-75.1]
JD-2000	788 [627-946] ^a	Practically Non-Toxic	90.5 ^e [76.1-108]

¹Toxicity classification per USEPA 2010 applied to results of present study

^aEstimated by linear regression method

^bEstimated by Spearman-Kärber method

^cEstimated by binomial method

^dValues as reported in NCP Product Schedule documentation by manufacturer

^eClassified as slightly toxic according to values provided in NCP Product Schedule

Table 2. Results of *Menidia* 96-hr static acute toxicity tests with eight dispersants. LC50 values (ppm), 95% confidence intervals [in brackets] and the toxicity classification of dispersant LC50s derived in the present study. NCP Product Schedule listing of dispersant LC50s and 95% confidence intervals [in brackets] shown in right column for comparison.

Dispersant	This Study LC50 (ppm) [95% CI]	Toxicity Category ¹	NCP Product Schedule LC50 (ppm) [95% CI] ^d
Dispersit SPC 1000	2.9 [2.5-3.2] ^b	Moderately Toxic	3.5 [3.1-4.0]
Nokomis 3-F4	19 [16-21] ^b	Slightly Toxic	29.8 [24.0-35.4]
Nokomis 3-AA	19 [17-21] ^b	Slightly Toxic	34.2 [29.2-37.95]
ZI -400	21 [18-23] ^b	Slightly Toxic	31.8 [28.7-35.1]
Saf-Ron Gold	44 [41-47] ^b	Slightly Toxic	29.4 [25.2-34.3]
Sea Brat #4	55 [49-62] ^b	Slightly Toxic	30.0 [±16.2]
Corexit 9500A	130 [122-138] ^b	Practically Non-Toxic	25.2 ^e [13.6-46.6]
JD-2000	>5,600	Practically Non-Toxic	407 [330-501]

¹Toxicity classification per USEPA 2010 applied to results of present study

^bEstimated by Spearman-Kärber method

^dValues as reported in NCP Product Schedule documentation by manufacturer

^eClassified as slightly toxic according to values provided in NCP Product Schedule

Appendix A

Test parameter	Specified in SubPart J Appendix C (USEPA 1997)	Method used in present study, and specified in USEPA 2002
Photoperiod and light intensity	*24 hr light *higher intensity light	*16 hr light/8 hr dark *Moderate intensity light
Glassware cleaning	*Hexane immersion	*Acetone rinse
Reference toxicant test	*Two species simultaneously	*Staggered tests
Rangefinder tests	*Prior to definitive test	*Use NCP data to define test concentrations
Mysid age	*5-7 day old larvae	*1 to 6 day old; all within 24 hr same age
Toxicant stock solution preparation for mysid test	*Blender 10,000 rpm *gas tight syringes	*Top stirring at 70% vortex *graduated glass pipettes
Mysid test solution mixing	*no specification	*short term gentle mixing following stock addition
Mysid additions to test chambers	*no specification	*impartial, two at a time
<i>Menidia</i> age	*7 day old larvae	*9-14 day old, all within 24 hr same age
<i>Menidia</i> test solution mixing	*test jars on shaker platform	*same procedure as for mysids
Dilution Water	*Natural Seawater Preferred	*Salinity adjusted, 20 μ m filtered natural seawater

May 20, 2010

Dispersant Monitoring and Assessment Directive – Addendum

This is an addendum (Addendum 2) to the Dispersant Monitoring and Assessment Directive issued on May 10, 2010, and Addendum 1 issued on May 14, 2010 by the U.S. Coast Guard (USCG) and the Environmental Protection Agency (EPA) to BP. The requirements in this Addendum 2 apply to Parts 1 and 2 of the May 10, 2010 Directive and are in addition to the requirements of that Directive. BP shall commence Parts 1 and 2 requirements before subsurface application of dispersant is initiated and continues the Parts 1 and 2 requirements, Addendum 1, and this Addendum 2 until cancelled or modified by the USCG and EPA.

Alternative Dispersant additional Requirements:

1. Sampling of dispersant/oil and oil-only waters must be continued per the Directive, and in addition, baseline data of waters without direct application of dispersant or oil shall also be collected by BP. Monitoring of subsurface dispersant application by BP shall be performed from a vessel capable of performing all requirements of the May 10, 2010, Dispersant Monitoring and Assessment Directive and Addendum 1 on each day that dispersant is applied. As used in this Addendum 2, a "day" shall mean a calendar day.
2. Within 24 hours of the issuance of this Addendum 2, BP shall identify to the FOSC and the EPA RRT Co-chair for EPA's and the FOSC's approval, one or more approved dispersant products from the National Contingency Plan Product Schedule that are available in sufficient quantities, are as effective at dispersing the oil plume, and have a toxicity value less than or equal to 23.00 ppm LC50 toxicity value for *Menidia* or 18.00 ppm LC50 for *Mysidopsis*, as indicated on the NCP Product Schedule (http://www.epa.gov/oem/content/ncp/tox_tables.htm). The less toxic dispersant product(s) shall be used by BP for surface application and subsurface application as directed by the FOSC. Within 72 hours after submitting the list of alternatives, and after receiving EPA approval, BP shall immediately use only the approved alternative dispersant. Should BP not be able to identify alternative dispersant products, BP shall provide the FOSC and EPA RRT CO-Chair a detailed description of the products investigated, the reason the products did not meet the standards described above. Availability shall be based on existing stockpiles of dispersants, the estimated time to begin and aerial and subsurface application, time for manufacturing, shipping, and warehousing.
3. The effectiveness of the dispersant in subsurface application shall be determined as specified in Directive 1 Part 1, and Part 2. Dispersant application can be applied subsurface if, and only if, daily monitoring is performed.
4. BP shall provide 48 hours advanced notice of departure and trip duration timelines of the monitoring vessel to the FOSC and the EPA RRT Co-chair.
5. Monitoring data on the use of the less toxic dispersant product(s) shall be reported by BP to the FOSC and the EPA RRT Co-chair on a daily basis. This reporting shall include a sample tracking table. Daily data reports shall thereafter be provided by BP to the FOSC and the EPA RRT Co-chair as soon as practicable on the day following use of the less toxic dispersant product(s) by BP, but in no event later than 24 hours after use.

May 26, 2010

Dispersant Monitoring and Assessment Directive - Addendum 3

Reduction in Use of Dispersants. BP shall implement measures to limit the total amount of surface and subsurface dispersant applied each day to the minimum amount possible. BP shall establish an overall goal of reducing dispersant application by 75% from the maximum daily amount used as follows:

a. Surface Application. BP shall eliminate the surface application of dispersants. In rare cases when there may have to be an exemption, BP must make a request in writing to the FOSC providing justification which will include the volume, weather conditions, mechanical or means for removal that were considered and the reason they were not used, and other relevant information to justify the use of surface application. The FOSC must approve the request and volume of dispersant prior to initiating surface application.

b. Subsurface Application. BP shall be limited to a maximum subsurface application of dispersant of not more than 15,000 gallons in a single calendar day.

Application of dispersant in amounts greater than specified in this Addendum 3 shall be in such amounts, on such day(s) and for such application (surface or subsurface) only as specifically approved in writing by the USCG Federal On-Scene Coordinator (FOSC).

June 30, 2010

Dispersants Toxicity Testing – Phase I Questions and Answers

Q1. Why is EPA only testing eight out of the total 14 dispersants on the National Contingency Plan Product Schedule?

EPA chose eight dispersants (Dispersit SPC 1000; Nokomis 3-F4; Nokomis 3-AA; ZI-400; SAF-RON GOLD; Sea Brat #4; Corexit 9500 A; JD 2000) from the dispersants listed on the National Contingency Plan Product Schedule based on three criteria: 1) lower toxicity of the dispersant or of the dispersant when mixed with oil; 2) availability of sufficient quantities to respond to the Gulf spill; and 3) immediate availability of samples for testing.

Q2. What toxicity tests are being conducted to determine the least toxic dispersants?

A: EPA is conducting several toxicity tests to provide independent scientific information about these eight dispersants. Three types of testing results on the dispersants alone are available:

- 1) Potential endocrine activity: Some of the dispersants include chemicals called nonylphenol ethoxylates (NPE). NPE breaks down in the environment to nonylphenol (NP) which is a substance that could potentially cause endocrine disruption.
- 2) Degree each is toxic to living cells – cytotoxicity: EPA used *in vitro* assays to test the degree to which these eight dispersants are toxic to various types of mammalian cells and the potential for each dispersant to exhibit endocrine activity.
- 3) Acute toxicity to shrimp and small fish -Acute toxicity tests are used to determine lethal concentrations of the test chemicals.

Currently underway are acute toxicity tests on Louisiana sweet crude oil alone and combinations of sweet crude oil with each of the dispersants for each test species.

The companies who manufacture the different types of oil spill dispersants already tested both the toxicity and the effectiveness of each of these dispersants and submitted results to EPA for listing their product on the National Contingency Plan Product Schedule. Although these industry-submitted test results provide guidance, the tests were conducted on the dispersants by different laboratories and on the dispersants mixed with No. 2 fuel oil which is not the type of oil in the Gulf. EPA wanted to conduct its own toxicity tests in one laboratory under EPA oversight for better comparative analysis and to test the dispersants mixed with the oil from the Gulf.

Q3: What tests did EPA use to assess potential endocrine activity?

A: Some of the dispersants include chemicals called nonylphenol ethoxylates (NPE). NPE breaks down in the environment to nonylphenol (NP) which is a substance that could potentially cause endocrine disruption. Endocrine disruption can lead to defects in fetal development or can impair reproductive health in humans and aquatic species. The degree to which the eight types of oil spill dispersants are toxic to various types of cells is one good measure for estimating how much of the dispersant it would take to cause cell death. The more dispersant it takes to cause cell death, the less toxic the dispersant.

Estrogen and androgen receptors are proteins in the body that interact with the hormones estrogen and testosterone and respectively control development and function of the female (estrogen) and male (androgen) reproductive organs.

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Q3a: What tests did EPA use to assess the degree each dispersant is toxic to living cells (cytotoxicity)?

A: In vitro assays are fast, often automated chemical screening tests that assess the potential for a chemical to affect specific biological processes that could impact human health and the environment.

Q4: What tests did EPA use to assess acute toxicity to shrimp and small fish?

A: Acute toxicity tests are used to determine lethal concentrations of the test chemicals. The acute toxicity to shrimp and fish was determined using a standardized 48-hour mysid shrimp and a 96-hour small fish test to evaluate the potential toxicity of the dispersant. Established testing procedures are specified for both the mysid shrimp and small fish. Both species live in the bays and estuaries of the northern Gulf of Mexico and are commonly used in toxicity tests. These two tests are required by EPA to list a dispersant on the **National Contingency Plan Product Schedule**. The test protocol exposes mysid shrimp or small fish to a range of dispersant concentrations and dispersant-oil mixtures separately in the laboratory. Toxicity is determined by comparing the survival of the mysid shrimp or small fish exposed to the dispersants or dispersant-oil mixtures to survival of these organisms to a standard reference chemical.

The aquatic organisms used as test species are small mysid shrimp, *Americamysis bahia*, and a small fish, *Menidia beryllina*. Survival of the animals exposed to multiple concentrations of the dispersants and oil-dispersant mixtures will be determined for each species. The concentration lethal to 50 percent of the test animals is calculated and compared between dispersants and between the toxicities of the oil-dispersant mixtures to determine the most and least toxic chemicals and combinations.

Q5: What are the dispersant test results for potential endocrine activity, cytotoxicity and acute toxicity to shrimp and small fish?

A: While the dispersant products alone – not mixed with oil – have roughly the same effects, JD-2000 and Corexit 9500 proved to be the least toxic to small fish, and JD-2000 and SAF-RON GOLD were the least toxic to the mysid shrimp.

None of the eight dispersants tested displayed biologically significant endocrine disrupting activity, with the exception of a weak response for two of the dispersants (Nokomis 3-F4 and ZI-400) in one of the tests. This estrogenic result is likely not of biological significance. Cell death (degree the dispersant is toxic to living cells) was observed in some tests at concentrations above 10 parts per million. The endocrine and the cytotoxicity screening were conducted at dispersant concentrations from 0.001 parts per million up to 10,000 parts per million.

None of the dispersants triggered cell death at the likely concentrations of dispersants expected in the Gulf.

The next phase of EPA's testing will assess the acute toxicity of multiple concentrations of Louisiana Sweet Crude Oil with each of the eight dispersants for two test species. The shrimp and small fish in the tests were exposed to a range of concentrations of each dispersant bracketing the median lethal concentrations listed in the NCP Product Schedule. Median lethal

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concentrations indicate how much it takes to kill half of the sample of fish and mysid shrimp being tested. The dispersant concentrations tested ranged from one part per million (ppm) to more than 5,000 ppm for some dispersants.

Toxicity Testing - Question & Answers&As

Q6: Why are toxicity tests used?

A: Toxicity tests are used to determine the potential adverse effects of chemicals on humans and other organisms. Acute toxicity tests are used on different species to determine lethal concentrations. Chronic toxicity tests can be used to determine sublethal chemical concentrations such as those adversely affecting reproduction, growth and developmental processes as a result of long-term (chronic) exposure. In vitro assays are used to screen a large quantity of chemicals using many tests to prioritize which chemicals have the potential to be the most toxic.

Q7: What are "In Vitro Assay" screenings and why are they used?

A: In vitro assays are fast, often automated chemical screening tests that do not use live animals to assess the potential of a chemical to affect specific biological processes that may impact human health and the environment. On average, it would take a researcher eight hours a day, five days a week, for 12 years to do these assays. With computer we can do these tests and get results in three days. These types of tests are used regularly in the pharmaceutical industry to study the effects of drugs and medications and are also used to assess environmental chemicals.

Traditional chemical toxicity testing (typically animal tests in the lab) are time consuming and expensive. In vitro assay screening provides fast, often automated screening results for assessing the potential of a chemical to affect human health and the environment. This type of screening can be used to prioritize which chemicals need further toxicity testing using animals. Animal tests on one chemical can cost millions of dollars. In comparison, numerous in vitro tests can be run on a chemical for about \$20,000.

Assistant Administrator Paul Anastas
Dispersant Testing Release
June 30, 2010

As prepared for delivery.

Thank you all for joining us. Today we are releasing the data gathered from our first round of toxicity testing of eight oil dispersants. This testing was prompted by Administrator Jackson's direction that BP and EPA obtain further data on all approved and available dispersants, including Corexit 9500, the product currently in use.

Administrator Jackson has said many times that the decision EPA and the Coast Guard made to authorize the use of dispersants was a difficult choice – but one suited to the emergency we're facing. With a spill of this size and scope, dispersants are useful in breaking up the oil and preventing its spread – particularly to fragile wetlands.

That approval has come with strict conditions. We have limited the daily amount of subsea use. We have required strict monitoring of environmental conditions in the areas of application. And in the month after EPA and the Coast Guard directed BP to ramp down dispersant use, the volume applied dropped nearly 70 percent from peak usage. That policy does not change, even with these initial data.

EPA has also insisted on transparency. Administrator Jackson helped persuade NALCO, the company that manufactures Corexit, to release the Confidential Business Information claims and publicly disclose details about the ingredients of their dispersant. EPA has provided a broad range of information on dispersants and other issues on our website <http://www.epa.gov/bpspill>. The next step in the push for transparency is the testing we're releasing today.

Let me be clear: this is the **first** round of data. I know many of you are interested to hear if this testing means EPA will order BP to switch dispersants. We are not making any such recommendation at this time. We have additional testing to do.

What today's data are showing is that, in the tests we performed, all of the dispersants are roughly equal in toxicity, and generally less toxic than oil. None of the eight dispersants tested displayed biologically significant endocrine disrupting activity.

JD-2000 and Corexit 9500, the product currently in use, proved to be the least toxic to small fish, while JD-2000 and SAF-RON GOLD were the least toxic in the tests on mysid shrimp.

Finally, internal modeling results show that the dispersant constituents are expected to biodegrade in weeks to months, rather than remaining in the ecosystem for years as oil might.

Let me be clear about another point as well: this first round of testing studied specific effects under specific conditions. These data provide information on only some of the variables that we must consider. We are going to need more testing to get a full picture of dispersant impacts, and make any determination as to whether one product ranks better or worse than another under all of the conditions of its use.

The next phase of EPA's testing will look at the acute toxicity of multiple concentrations of Louisiana Sweet Crude oil alone and combinations of Louisiana Sweet Crude oil with each of the eight dispersants for two test species. Additional studies are underway to better understand endocrine activity.

We need more data before deciding whether it makes sense to change dispersants. But our ultimate goal in all of this is to reach a point where dispersants are no longer necessary – to fully phase out their use and rely on oil collection, burning, skimming and other methods to protect our Gulf and our shorelines. It's important to remember that oil

is enemy number one in this crisis. So we will continue testing, and we will be sharing more information as soon as we have it. Meanwhile, we are doing everything we can as part of this historic response.

Analysis of Eight Oil Spill Dispersants Using *In Vitro* Tests for Endocrine and Other Biological Activity

June 30, 2010

U.S. Environmental Protection Agency
Office of Research and Development

Executive Summary

The U.S. Environmental Protection Agency's Office of Research and Development was asked to evaluate the cytotoxicity and potential for interaction with the androgen and estrogen receptors (AR, ER) of eight oil spill dispersants being used, or could be considered for use, in the Gulf of Mexico. These are Corexit 9500 (the current product being used), DISPERSIT SPC 1000, JD 2000, Nokomis 3-F4, Nokomis 3-AA, SAF-RON GOLD, Sea Brat #4, and ZI-400. To address this request, ORD staff and outside collaborators carried out a number of separate studies that were run using *in vitro* (cell-based) assays. A total of 8 cytotoxicity assays, 3 AR agonist assays, 1 AR antagonist assay and 4 ER agonist assays were run on the 8 dispersants, plus reference compounds. Tests were run across a wide range of dispersant concentrations (0.001 to 10,000 parts per million, or ppm). Two dispersants showed a weak signal in one of the four ER assays, but integrating over all of the ER and AR results these data do not indicate that any of the eight dispersants display biologically significant endocrine activity via the androgen or estrogen signaling pathways. All of the dispersants showed cytotoxicity in at least one cell type at concentrations between 10 and 1000 ppm. Both JD 2000 and SAF-RON GOLD tend to be less cytotoxic than the other dispersants. Likewise, DISPERSIT SPC 1000 tends to be more cytotoxic than the other dispersants in the cell-based assays.

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Introduction / Background

The Deepwater Horizon oil spill has led to the use of large amounts of dispersant as part of the integrated approach dealing with the oil spill. Given this fact, questions have arisen about the toxicity of the chemicals used as dispersants themselves. EPA's Office of Research and Development (ORD) was asked to carry out rapid studies to provide information on the potential for toxicity of eight commercially available dispersants. Because some of the dispersants reportedly include nonylphenol ethoxylates (NPEs) that can degrade to isomers of nonylphenol (NP), some of which are proven estrogenic compounds, the potential endocrine effects of the dispersants are of particular concern. For example, NPEs and NPs have been demonstrated to be endocrine disruptors in fish [1]. In response to the request ORD has undertaken a series of short-term *in vitro* studies to determine if any of the dispersants displayed estrogenic, androgenic or other endocrine activity.

ORD developed a strategy to address the questions of endocrine activity and relative toxicity as rapidly as possible. ORD scientists initiated several complementary studies of eight oil spill dispersants being used or considered for use in the Gulf. The issue was to provide some targeted information on the dispersants as quickly as possible. *In vitro* assays are well suited for that purpose. This work complements a study of whole animal toxicity in small fish and brine shrimp also being carried out by ORD. The results of that study are being simultaneously released with this report.

One set of studies used a set of mammalian *in vitro* reporter gene assays in estrogen-responsive and androgen-responsive cells [2, 3] run in-house at ORD laboratories in RTP, NC. Additional studies were conducted by two external labs (NIH Chemical Genomics Center [NCGC] and Attagene Inc.) to run mammalian *in vitro* reporter gene assays to measure androgen and estrogen-response activity. A panel of 74 assays against non-endocrine molecular targets was also included in the Attagene assays. The NCGC and Attagene assays are part of the EPA ToxCast program [4, 5]. All assays evaluated the eight dispersants Corexit[®] 9500, JD 2000[™], DISPERSIT SPC 1000[™], Sea Brat #4, Nokomis 3-AA, Nokomis 3-F4, ZI-400 and SAF-RON

GOLD. The performance of the assays was characterized by simultaneously running positive and negative control chemicals. Quantitative cytotoxicity measurements were carried out on each of the cell types used. All data analyses and interpretation were carried out by ORD staff.

It is important to note that positive results *in vitro* only demonstrate that a chemical is a potential endocrine disruptor and that follow-up tests will likely be needed in order to refine or confirm the endocrine activity. For example, effects seen *in vitro* may not be expressed *in vivo*, so additional studies would need to be conducted to verify the *in vitro* results and determine if the potential activity was displayed in whole animals and the dosage levels required to affect organisms.

Project Goals

1. Determine if any of the eight dispersants displayed estrogenic, androgenic or antiandrogenic activity *in vitro* using a variety of well characterized *in vitro* cell-based assays that utilize different approaches for detecting endocrine driven gene expression changes
2. Determine the dispersant concentration that induced cytotoxicity in multiple cell lines and derive an aggregate measure of cytotoxicity that could be use to rank order the chemicals and to compare with *in vivo* toxicity data obtained in aquatic test species.

Study Summary:

One part of the project was carried out by ORD researchers in partnership with the NIH Chemical Genomics Center [NCGC] and Attagene Inc. Two high throughput assay sets were run on the dispersants, a collection of reference chemicals for ER and AR activity, plus nonylphenol compounds. In addition to assays for AR and ER, this phase of the project produced data on a battery of other transcription factor assays which are part of multiplexed panels including AR and ER assays. Cytotoxicity was evaluated in three cell lines over a range of concentrations.

The other phase of the study was carried out in-house by ORD researchers using multiple assays [2, 3] to measure interaction between the eight dispersants plus reference chemicals and ER or AR. In particular, this work evaluated the eight dispersants for estrogen agonist activity in an estrogen-responsive transcriptional activation assays (ER-TA), for androgen agonist activity in two androgen-responsive transcriptional activation assays (AR-TA), MDA-kb2 and CV-1 assays and for androgen antagonist activity in the MDA-kb2 assay in competition with 1 nM Dihydrotestosterone (DHT). Cytotoxicity was evaluated in each assay at every concentration by both a biochemical assay which assessed metabolic perturbation and by a visual assessment of cytopathic effect on cell viability and morphology.

Chemicals

All assays evaluated eight commercially available oil spill dispersants that were obtained directly from the respective manufacturers. EPA chose these eight dispersants from the dispersants listed on the National Contingency Plan Product Schedule based on three criteria: 1) lower toxicity of the dispersant or of the dispersant when mixed with oil; 2) availability of sufficient quantities to respond to the Gulf spill; and 3) immediate availability of samples for testing. These included Corexit[®] 9500 (Nalco Inc., Sugarland TX), JD 2000[™] (GlobeMark Resources Ltd., Atlanta, GA), DISPERSIT SPC 1000[™] (U.S. Polychemical Corp., Chestnut Ridge, NY), Sea Brat #4 (Alabaster Corp., Pasadena, TX), Nokomis 3-AA (Mar-Len Supply, Inc., Hayward, CA), Nokomis 3-F4 (Mar-Len Supply, Inc., Hayward, CA), ZI-400 (Z.I. Chemicals, Los Angeles, CA) and SAF-RON GOLD (Sustainable Environmental Technologies, Inc., Mesa, AZ). All are liquid solutions. Further information on the dispersants, including the limited publicly available information on the composition of dispersants is given in **Appendix A.1**. The oil spill dispersants were tested *in vitro* at concentrations ranging from 0.01 to 1000 ppm in water (vol:vol).

The assays run by NCGC and Attagene included reference compounds recommended for validating ER /AR assays by ICCVAM (Interagency Coordination Committee on the Validation of Alternative Methods)[6] and the U.S. EPA[7]. A preliminary set of reference compounds was obtained from stocks at EPA facilities in RTP NC. Subsequently, additional samples were obtained from Sigma-Aldrich (St. Louis MO). Included in the reference chemicals are both straight chain and branched NP isomers and corresponding example NPEs. The reference chemicals are 17 β -Trenbolone (10161-33-8), 17 β -Estradiol (50-28-2), Atrazine (1912-24-9), Bisphenol A (80-05-7), Butylbenzyl phthalate (85-68-7), Dibutyl phthalate (84-74-2), Flutamide (13311-84-7), Linuron (330-55-2), 4-Nonylphenol (linear) (104-40-5), p,p'-DDE (72-55-9), p,p'-Methoxychlor (72-43-5), Procymidone (32809-16-8), Vinclozolin (50471-44-8), 2,4,5-T (93-76-5), Bicalutamide (90357-06-5), Cyproterone acetate (427-51-0), Genistein (446-72-0), 4-(tert-octyl), Phenol (140-66-9), 4-Hydroxytamoxifen (68392-35-8), 5 α -androstan-17 β -ol-3-one (521-18-6) and 4-Nonylphenol, (branched) (84852-15-3). The two nonylphenol ethoxylates are Tergitol NP-9 (127087-87-0) and Igepal CO-210 (68412-54-4). Reference chemicals (powder

form) were solubilized in DMSO to a final concentration of 20 mM. Further information, including lot and batch are given in **Appendix A.2**.

In the in-house ORD assays, a 17β -Estradiol (E2; 50-28-2) dose response was included on every plate in the ER-TA assay as a positive control. 4-Nonylphenol (branched) (84852-15-3; Fluka) and 17α -Trenbolone (Osaka Hayashi Pure Chemical Industries Ltd., CAS no. 80657-17-6, purity 99.9%) were also tested in the estrogen mediated assays. A dihydrotestosterone (DHT; Sigma Chemical; CAS 55206-14-9) dose response was included as a positive control on every plate in the AR-TA assays. The potent androgen, 17α -Trenbolone, was also tested in the androgen agonist assays. Dosing solutions of dispersants and reference compounds were prepared on-site under observation of a Quality Assurance manager. The assays used in the NHEERL assays have been demonstrated [2, 8] to give appropriate responses to known estrogenic or androgenic compounds.

Results

More detailed assay protocols and statistical analysis methods can be found in the Appendices, as well as a Quality Assurance (QA) Statement.

Androgen Receptor Agonist Activity

AR Agonist Assay 1 - Multiplexed reporter transcription unit (RTU) assay

Method Summary: This assay is part of a multiplexed reporter gene panel run by Attagene Inc. (RTP, NC), under contract to the U.S. EPA (Contract Number EP-W-07-049). This assay consists of 48 human transcription factor DNA binding sites transfected into the HepG2 human liver hepatoma cell line as previously described[9]. This *trans* assay employs a mammalian one-hybrid assay consisting of an additional 25 RTU library reporting the activity of nuclear receptor (NR) superfamily members. The human ligand-binding domain of each nuclear receptor was expressed as a chimera with the yeast GAL4 DNA-binding domain that activated in *trans* a 5XUAS-TATA promoter, which regulated the transcription of a reporter sequence unique to each NR RTU. To ensure the specificity of detection, each individual *trans*-RTU system including both receptor and reporter gene was separately transfected into suspended cells followed by pooling and plating of the transfected cells prior to screening. The *trans* assay evaluates changes in activities of exogenous, chimeric NR-Gal4 proteins. This particular assay evaluated transcription for the Androgen receptor, and uses the code ATG_AR_TRANS. Additional detail of the method is provided in the **Appendix B.1**. Concentration-response titration points for each compound were fitted as described in **Appendix C**. For this analysis, there were either 4 replicates in 16 concentrations, except for SAF-RON GOLD which was only tested in 2 replicates and 8 concentrations.

Results: No activity was seen for any of the dispersants

AR Agonist Assay 2 - AR beta-lactamase Assay

Method Summary: This assay was run at the NIH Chemical Genomics Center (NCGC; Rockville, MD) in collaboration with EPA as part of the Tox21 collaboration[10]. A beta-lactamase reporter-gene cell-based assay [GeneBLAzer[®] AR-UAS-bla-GripTite[™] assay developed by Invitrogen] was used to measure AR ligand signaling. AR-UAS-bla-GripTite[™] HEK 293 cells (AR *bla* cells) were used with assay medium containing 10% dialyzed FBS, 0.1 mM NEAA and 1 mM sodium pyruvate. The assay was performed in clear bottom black Greiner 1536-well plates. R1881, a synthetic androgen agonist, was used as a positive control in the screen. Library compounds were measured for their ability to either stimulate or inhibit the reporter gene activity. Compounds were screened in a titration series in 1536-well format. The fluorescence intensity (405 nm excitation, 460/530 nm emission) was measured using an EnVision plate reader. Data was normalized relative to R1881 control (40 nM, 100%, for agonist mode and 10 nM, 0%, for antagonist mode), and DMSO only wells (basal, 0% for agonist mode and -100% for antagonist mode). Additional detail of the method is provided in the **Appendix B.2**. Concentration-response titration points for each compound were fitted as described in **Appendix C**. For this analysis, there were 8-10 replicates in 24 concentrations.

Results: The only dispersant that showed any activity in any of the AR assays was JD 2000, which was active in both the NCGC ER and AR agonist and antagonist assays in all runs with AC50 values ranging from 100-270 ppm (AR) and 82-120 ppm (ER). There was no apparent cytotoxicity in any of the cell line for JD 2000 (see results below). The EMax values for JD 2000 in all of these assays were significantly greater than the values for positive control chemicals, and in the antagonist assays, this dispersant looked like a “super-activator” rather than an antagonist. All of this data taken together indicates strongly that some non-specific activation is occurring that is independent of ER or AR. We have found previously that compounds identified as promiscuous “super-activators” in multiple beta-lactamase reporter gene assays with a narrow potency range (a <3-fold difference in potency is within the experimental variations of these assays) are mostly auto fluorescent (R Huang, unpublished data). Thus, the activity observed for JD 2000 is likely an artifact of the beta-lactamase assay format. Preliminary results from three additional beta-lactamase assays for non-steroid receptor targets all showed the JD 2000 “super-

activation". Considering the totality of the data, we conclude that JD 2000 does not exhibit ER or AR transactivation activity. To further confirm that this JD 2000 activity is non-specific and not due to ER or AR activation, we are running several follow-up assays with NCGC: known antiestrogens and antiandrogens are being used to show that JD 2000 activity is not suppressed; and we will complete our analysis of results for the the three non-steroid receptor beta-lactamase assays are being run with JD 2000 to show that this non-specific activity occurs independent of ER and AR.

AR Agonist Assay 3 - MDA-kb2 Androgen-responsive transcriptional activation assay

Method Summary: This assay, run in-house by NHEERL researchers, utilized MDA-kb2 cells[2]. These cells contain endogenous human androgen receptor capable of inducing transcription of an androgen responsive gene (AR-TA). This assay employs a luciferase gene driven by the androgen responsive MMTV promoter which has been stably integrated into the cells. When androgen mimicking compounds (i.e. compounds that act as androgen agonists) are present, these cells produce luciferase in a concentration proportionate to the efficacy of the androgen mimic. Nine concentrations of each dispersant were tested for agonist activity. Each concentration was evaluated in a total of eight replicates (two independent evaluations with four replicates per assay). The first dilution of each sample was a 1:100 dilution (i.e. 0.01 dilution or 10,000 ppm) of the dispersant in cell culture medium followed by eight additional 10-fold serial dilutions. Additional detail of the method is provided in the **Appendix F**.

Results: The ability of the eight dispersants to stimulate luciferase expression in this cell line was compared to DHT. The DHT positive control dose induced luciferase expression in MDA Kb2 cells in a precise and reproducible manner within and among the plates (**Figure 1**. DHT and 17 α -Trenbolone data in MDA Kb2 cells). None of the eight dispersants displayed any potential androgenicity (i.e. did not simulate luciferase induction) at any concentration in the MDA Kb2 cell line (**Figure 2** dispersant results in MDA Kb2 cells). In fact, all the dispersants displayed significantly reduced luciferase levels due to cytotoxicity at high dispersant concentrations. The synthetic androgen 17 α -Trenbolone acted as a full androgen agonist at relatively low concentrations.

**MDA Kb2 cell DHT
DATA FROM FIVE PLATES**

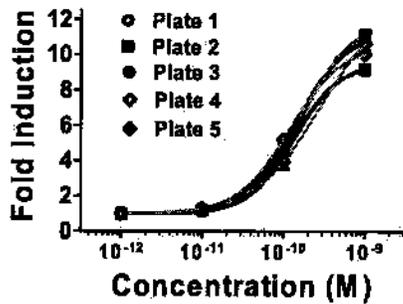


	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
LogEC50	-9.843	-8.776	-8.871	-9.553	-9.923

**CV1 DHT
DATA FROM 5 PLATES**

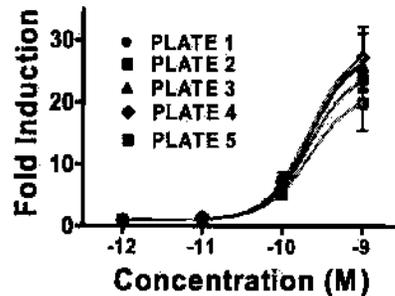
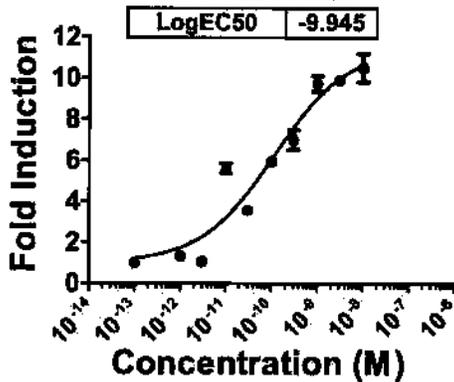


	PLATE 1	PLATE 2	PLATE 3	PLATE 4	PLATE 5
LogEC50	-9.867	-9.558	-9.726	-9.863	-9.878

**17 α Trenbolone
MDA Kb2 CELLS**



**Hydroxyflutamide a known antiandrogen used
as a positive control for inhibition of androgen action**

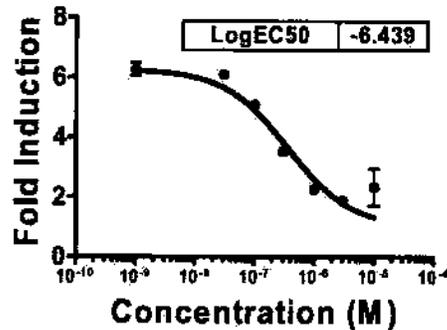


Figure 1: Included are, nonlinear regression plots of the effects of the reference androgen dihydrotestosterone (DHT) in two androgen sensitive cell lines (MDA Kb2 upper left and CV1 upper right), stimulatory effects of the synthetic androgen found in some aquatic systems in MDA Kb2 cells (lower left) and antagonism of the 1 nM DHT by the antiandrogenic drug hydroxyflutamide in MDA Kb2 cells. Data are expressed as fold over the media plus the ethanol control value. The X axis is in log scale. Values are means plus or minus standard errors of the mean.

Assessment of Potential Androgenicity in MDA Kb2 cells. The dispersants did not induce luciferase expression in an androgenic manner

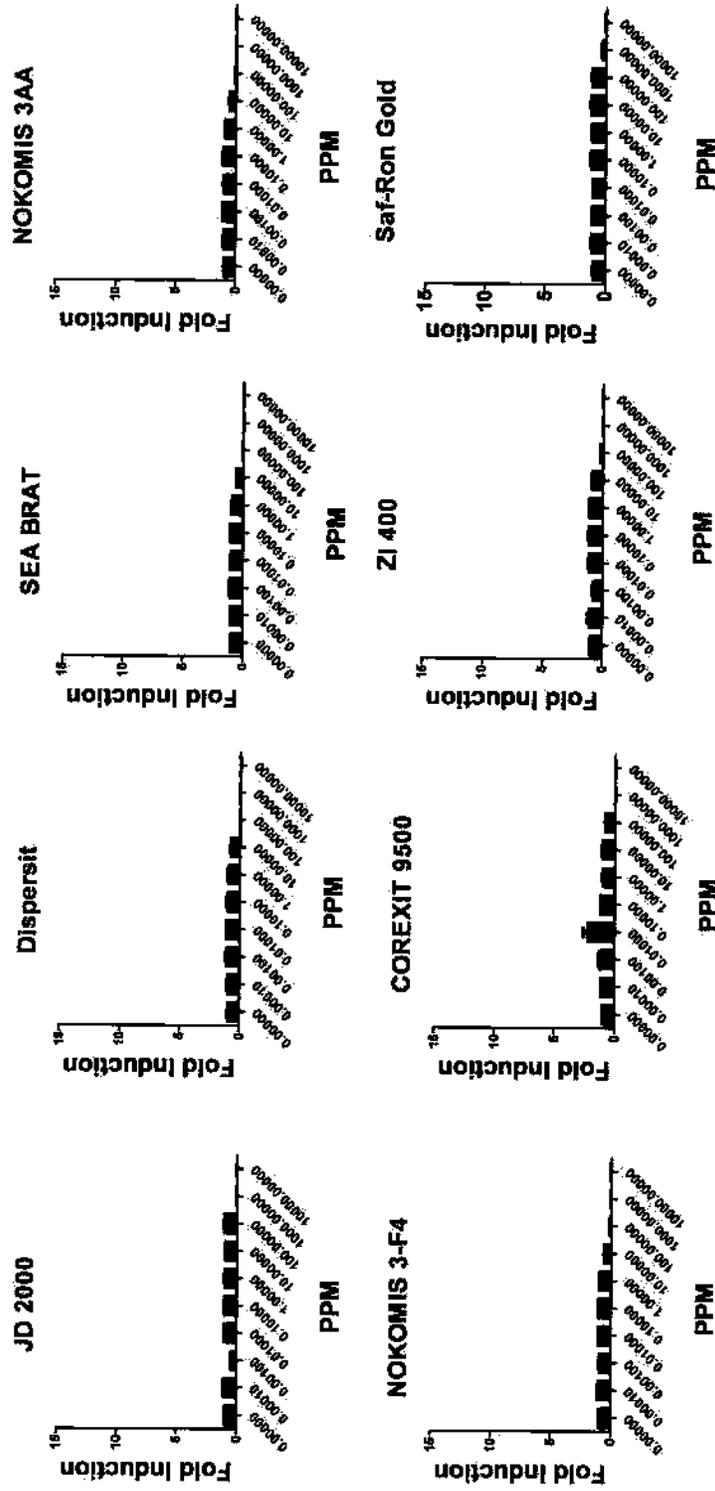


Figure 2: Assessment of the potential androgenic activity of the eight dispersants in MDA Kb2 cells. Data are expressed as fold over the media plus ethanol vehicle control. Values are means plus or minus standard errors of the mean. Dispersants did not stimulate luciferase induction over the control fold value (control fold =1).

AR Agonist Assay 4 - CV-1 transient transcription assay

Method Summary: This assay run in-house by NHEERL is similar to the MDA-kb2 in that it also assesses the ability of a compound to mimic an androgen. This assay, however, uses CV-1 cells which do not express either endogenous androgen or estrogen receptors. In contrast to the MDA-kb2 assay, both the androgen receptor and the androgen responsive MMTV promoter- luciferase reporter constructs are introduced into the CV-1 cells for each assay via transient transfection. Nine concentrations of each dispersant were tested for agonist activity in both AR-TA assays. Each concentration was tested in quadruplicate. The first dilution of each sample was a 1:100 dilution (i.e. 0.01 dilution or 10,000 ppm) of the dispersant in cell culture medium followed by eight additional 10-fold serial dilutions. Method details are provided in the **Appendix F**.

Results: Similar to the results of the MDA-kb assays, DHT induced precise and reproducible effects on luciferase expression within and among the plates (Figure 1) and none of the eight dispersants displayed any potential androgenicity (i.e. did not simulate luciferase induction) at any concentration in the CV-1 assay (Figure 3). In fact, all the dispersants significantly reduced luciferase level due to cytotoxicity at high concentrations.

Assessment of Potential Androgenic activity in CV-1 cells. The dispersants did not induce luciferase expression in an androgenic manner

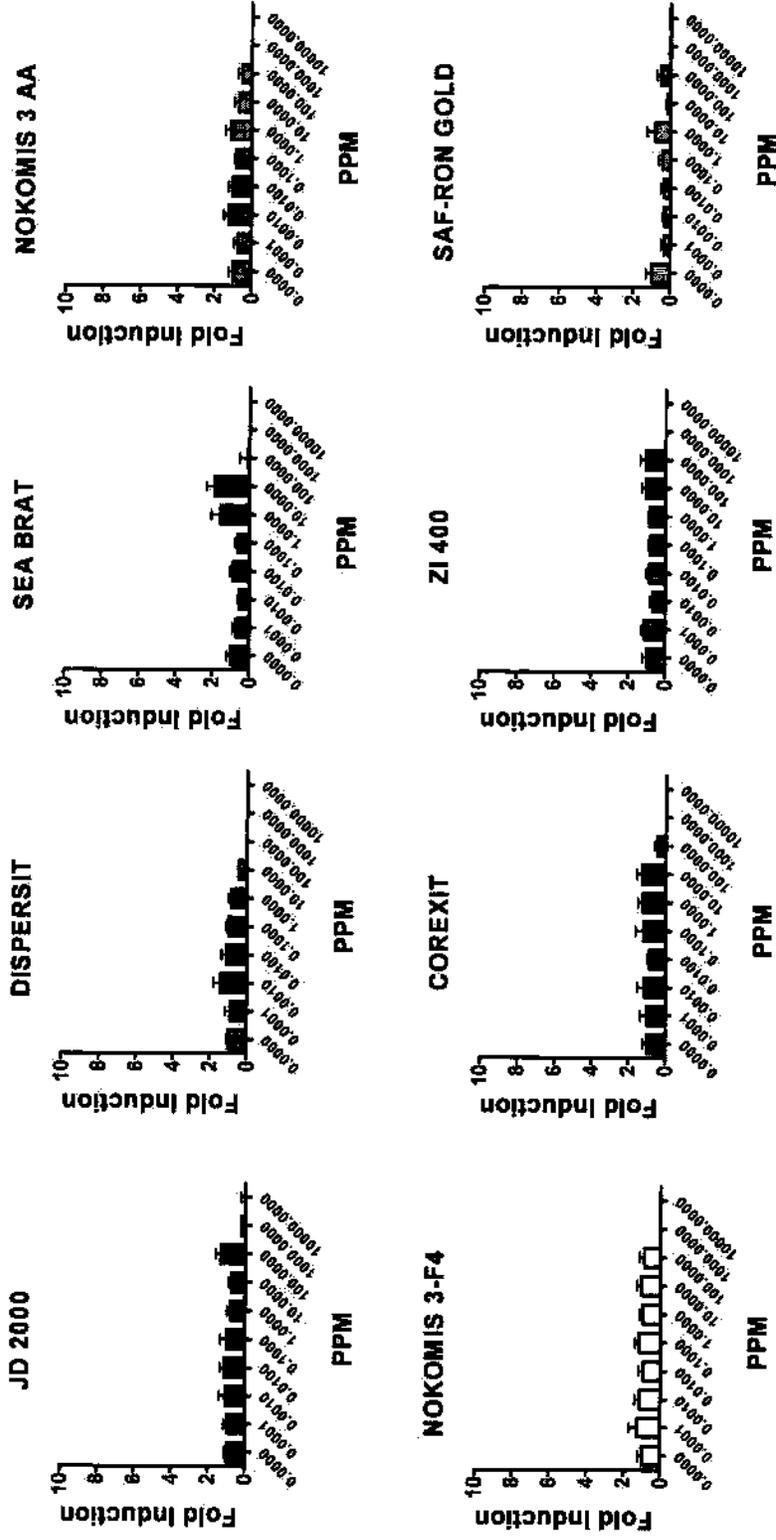


Figure 3: Assessment of the potential androgenic activity of the eight dispersants in CV-1 cells. Data are expressed as fold over the media plus ethanol vehicle control. Values are means plus or minus standard errors of the mean. Dispersants did not stimulate luciferase induction over the control fold value (control fold =1).

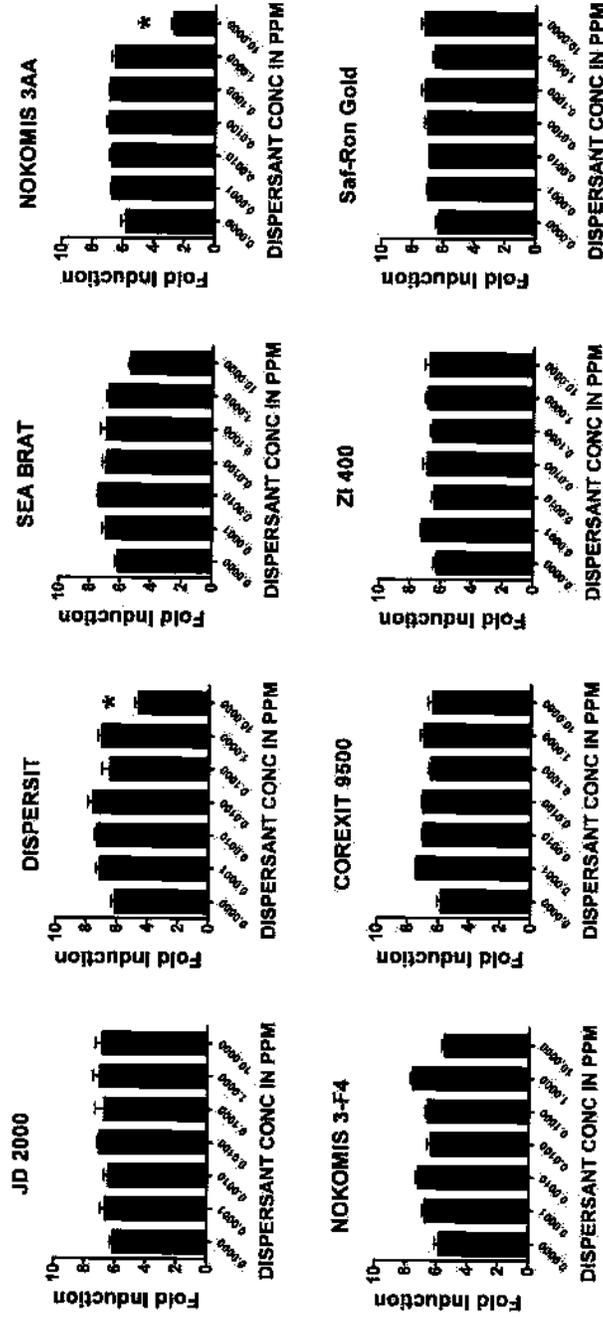
Androgen Receptor Antagonist Activity

AR Antagonist Assay 1 - MDA-kb2 Androgen-responsive transcriptional activation assay in antagonist mode

Method Summary: The eight dispersants were also evaluated for antagonist activity in the MDA-kb2 cell line, run in-house by NHEERL researchers, by testing each dispersant in the presence of a near maximally stimulating concentration dihydrotestosterone (1 nM DHT). In the presence of an anti-androgen, the luciferase activity induced by DHT would be reduced proportionally to the concentration of the anti-androgen. A DHT concentration-response curve was included on each 96-well plate with the dispersants. The well-characterized antiandrogen hydroxyflutamide (CAS 80657-17-6) was run as a positive control (**Figure 1**). Six concentrations of each dispersant ranging from 0.0001 ppm to 10 ppm were tested for antagonist activity. Higher concentrations were not evaluated due to cytotoxicity seen in both MTT and CPE assays (discussed later in this document). Each concentration was tested in quadruplicate. Additional detail of the method is provided in the **Appendix F**.

Results: None of the eight dispersants displayed any potential antiandrogenicity (i.e. did not inhibit DHT-induced luciferase induction) at concentrations below 10 ppm (1E-5 dilution). At 10 ppm several of the dispersants reduced DHT induced luciferase activity, but the effects were significant (by ANOVA followed by a post hoc Dunnett's test) for the dispersants SPC 1000 and Nokomis 3-AA (**Figure 4**). As shown in **Figure 5a**, these two dispersants were the most toxic of the dispersants to MDA Kb2 cells so it is extremely unlikely that these effects represent competitive inhibition of DHT binding to the ligand binding domain of the androgen receptor. In contrast, hydroxyflutamide, used as a positive control, completely inhibited androgen-induced luciferase induction at concentrations about 1000 fold higher than of the concentration of DHT used in this assay.

Assessment of potential antiandrogenicity of Dispersants in MDA Kb2 cells. The dispersant did not compete with the 1 nM DHT in the assay and lower luciferase expression in an antiandrogenic manner



* indicates $p < 0.01$ by Dunnett's test following ANOVA

Figure 4: Assessment of the potential antiandrogenic activity of the eight dispersants in MDA Kb2 cells. Data are expressed as fold over the media plus ethanol vehicle control. Values are means plus or minus standard errors of the mean. DISPERSIT SPC 1000 and Nokomis 3-AA were the only dispersants that significantly reduced DHT-stimulated luciferase induction (DHT control fold about 6) an effect which we interpreted to result from cytotoxicity at 10 ppm, the highest concentration used in this assay.

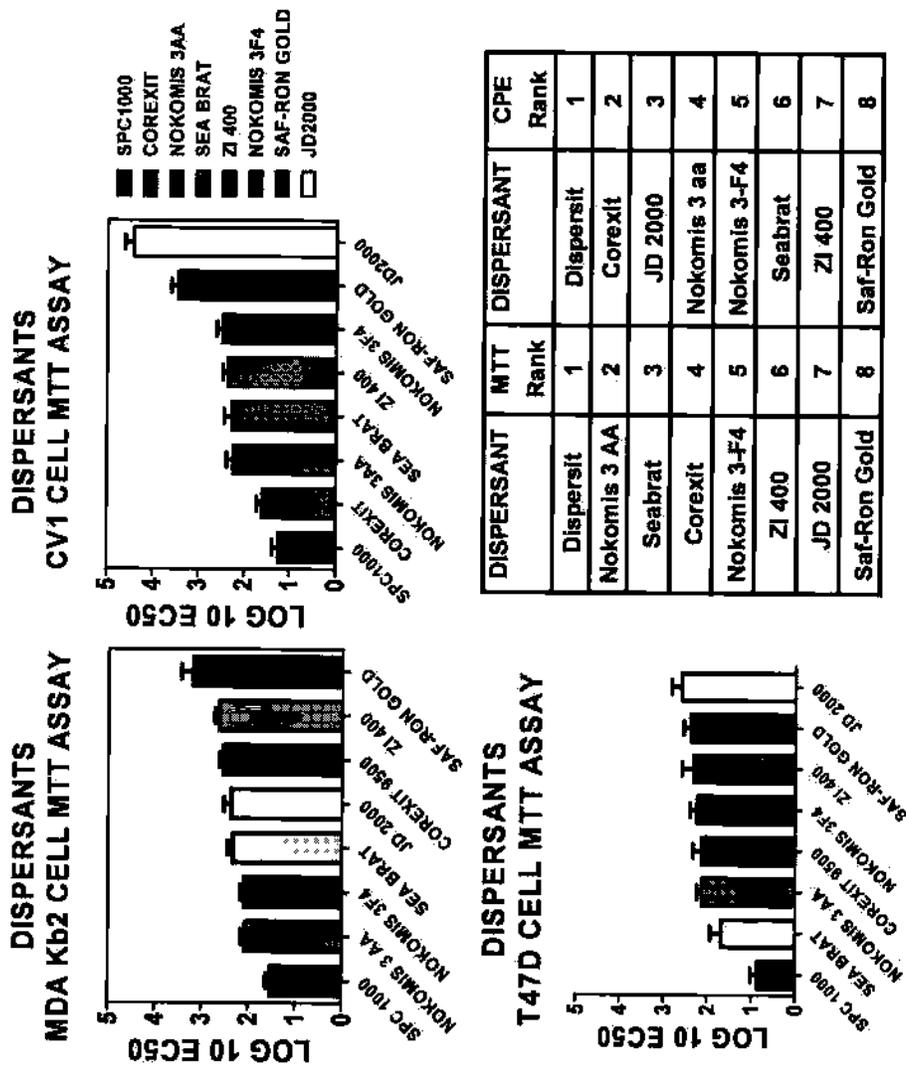


Figure 5: Summary of the Cytotoxic effects of the eight dispersants in the MTT and visual cytopathic examinations (CPE) in three cell lines. The histograms display the EC₅₀ values for a reduction in MTT levels (determined by nonlinear regression on Prism 5.0) for the MDA Kb2 (upper right panel), CV1 (upper right panel) and T47D Kbluc

(lower left panel) cells. In the table in the lower right panel, the overall potency of the dispersants in the MTT and CPE assays is shown, with a ranking of 1 being the most potent in inducing cytotoxicity and 8 being the least cytotoxic dispersant. DISPERSIT SPC 1000 was ranked as the most cytotoxic by both methods and SAF-RON GOLD is the least toxic of the eight dispersants. (The appendices contain additional details on this and the EC50 values are compared using a multiple range test).

Estrogen Receptor Agonist Activity

ER Agonist Assay 1 - Multiplexed reporter transcription unit (RTU) *trans* assay

Method Summary: This assay is part of a multiplexed reporter gene panel run by Attagene Inc. (RTP, NC), under contract to the U.S. EPA (Contract Number EP-W-07-049). This assay consists of 48 human transcription factor DNA binding sites transfected into the HepG2 human liver hepatoma cell line as previously described[9]. This *trans* assay employs a mammalian one-hybrid assay consisting of an additional 25 RTU library reporting the activity of nuclear receptor (NR) superfamily members. The human ligand-binding domain of each nuclear receptor was expressed as a chimera with the yeast GAL4 DNA-binding domain that activated in trans a 5XUAS-TATA promoter, which regulated the transcription of a reporter sequence unique to each NR RTU. To ensure the specificity of detection, each individual trans-RTU system including both receptor and reporter gene was separately transfected into suspended cells followed by pooling and plating of the transfected cells prior to screening. The *trans* assay evaluates changes in activities of exogenous, chimeric NR-Gal4 proteins. This particular assay evaluated transcription for the Estrogen receptor alpha, and uses the code ATG_ERa_TRANS. This assay was run in twice in separate weeks, and in each case, run in duplicate. For this analysis, there were either 4 replicates in 16 concentrations, except for SAF-RON GOLD which was only tested in 2 replicates and 8 concentrations. Additional detail of the method is provided in the **Appendix B.1**. Concentration-response titration points for each compound were fitted as described in **Appendix C**.

Results: We observed statistically significant ER activity in two of the dispersants in the Attagene *trans*-ER α assay (Nokomis 3-F4 and ZI-400), detailed in **Table 1**. **Figure 6** (bottom panels) shows the concentration-response curves for the two active dispersants, which have EMax (maximum efficacy) values of between 3 and 4. This is in contrast to 17 β -Estradiol (top left panel, blue curve), which has an EMax value of 20. The top right panel of the figure shows the corresponding reference curve for the *cis*-ERE assay, showing that 17 β -Estradiol only elicits a response about half of that seen in the *trans* assay. To help interpret these results, we

simultaneously analyzed their performance on a set of 19 reference chemicals recommended by ICCVAM[6] and EPA OPPT[7]. This analysis (detailed in **Appendix E**) shows that these assays perform well for both positive and negative predictive value. The *trans*-ER α assay correctly matched ICCVAM expectation for 15 of 17 reference chemicals, with one false positive and one false negative. A comparison of the *cis* and *trans* assays shows that the reference chemicals in the *cis* assay consistently produce EMax values about half of that seen in the *trans* assay. This would explain the absence of observable activity for these dispersants in the *cis* assay, because we do not consider curves with EMax values below 2. The other curves in the bottom panels of **Figure 6** show data for NP and NPE compounds, described below.

Chemical	AC50 (ppm)	EMax	R ²	p-value
Nokomis 3-F4	16	3.9	0.65	0.00017
ZI-400	25	3.4	0.68	0.0041

Table 1: Summary results for the Attagene *trans*-ER α assay for the positive dispersants. EMax: maximal fold change. AC50: concentration at which 50 of maximal activity is seen.

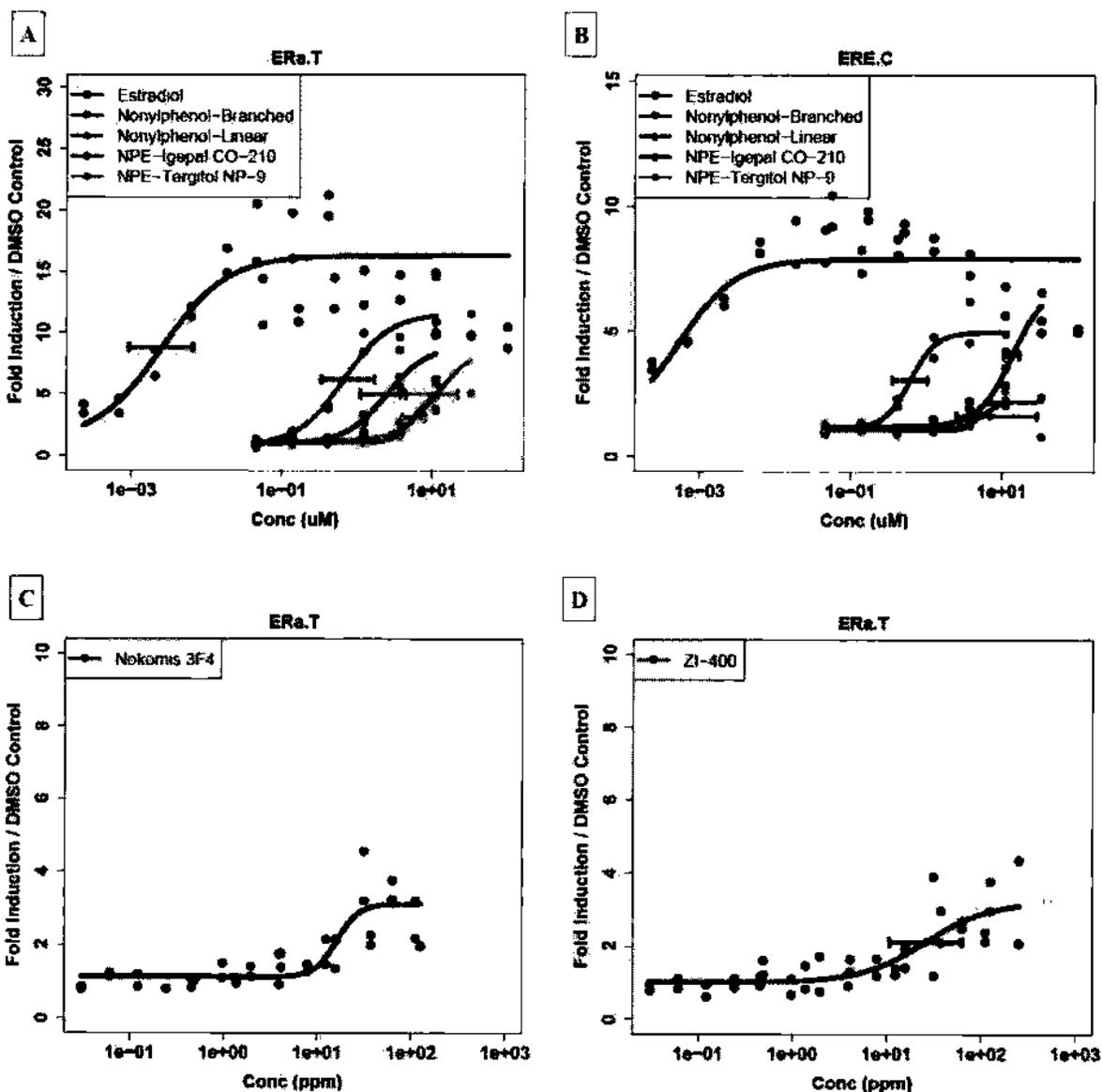


Figure 6: Concentration-response curves for the E2, NP and NPE compounds, and the two dispersants showing activity in Attagene *trans*-ER α assay. Top: E2 and the 4 NP / NPE compounds in the Attagene *trans*-ER α assay (left) and the Attagene *cis*-ERE assay (right). Bottom: ZI-400 and Nokomis 3-F4 in the Attagene *trans*-ER α assay. For the nonylphenol compounds, only two replicates were run.

Nonylphenol-related activity: It is known that some of the dispersants contain NPEs. Our initial hypothesis was that any estrogenic activity detected for the complex mixtures could be due to the

NPEs or to NP itself generated by *in situ* degradation of the NPE, or residual contamination from synthesis of the NPE. Consequently, we tested two nonylphenols (one linear and one branched, technical grade) and two commercial NPEs in the Attagene assays. **Table 2** shows the results of this analysis, and **Figure 6** shows the corresponding dose-response curves for the Attagene ER assays. From these data, one can see that these cell-based assays show ER activity for both the NPs and the NPEs. The branched, technical grade NP is the most potent, but the second most potent is the NPE Igepal CO-210. These data indicates that the presence of an NP or NPE in a mixture could give rise to ER activity such as was seen for the dispersants Nokomis 3-F4 and ZI-400. Public information (given in **Appendix A**) indicates that ZI-400 does in fact contain an NPE.

Chemical	Assay	AC50 (μ M)	R ²	EMax	p-value
4-Nonylphenol (linear) 104-40-5	<i>trans</i> -ER α	11	0.77	8.3	0.29
	<i>cis</i> -ERE	4.3	0.55	2.7	0.096
4-Nonylphenol (branched) 84852-15-3	<i>trans</i> -ER α	0.68	0.91	12	0.0049
	<i>cis</i> -ERE	0.61	0.092	5.4	4.9E-5
Tergitol NP-9 127087-87-0	<i>trans</i> -ER α	5.7	0.86	4.8	0.18
	<i>cis</i> -ERE	5.6	0.96	2.1	0.042
Igepal CO-210 68412-54-4	<i>trans</i> -ER α	2.5	0.89	8.5	0.19
	<i>cis</i> -ERE	14	0.96	6.5	2.1E-11

Table 2: Results of ER assays on NPs and NPEs.

To summarize this section, estrogen receptor (ER) activity was observed in two of the dispersants in the Attagene *trans*-ER α assay (ZI-400 and Nokomis 3-F4), although at relatively high concentrations and with low efficacy (EMax). We have also shown that NPs and NPEs are also active in the *trans*-ER α assay. Therefore, the activity in ZI-400 and Nokomis 3-F4 is suggestive of the presence of an NP or NPE as part of the mixture. We know that this is the case with ZI-400. The ER effect seen for these dispersants is weak, which is also suggestive of there being only a relatively small amount of NPE or some other estrogenic substance in the total mixture.

ER Agonist Assay 2 - Multiplexed reporter transcription unit (RTU) *cis* assay

Method Summary: This assay is part of a multiplexed reporter gene panel run by Attagene Inc. (RTP, NC), under contract to the U.S. EPA (Contract Number EP-W-07-049). This assay consists of 48 human transcription factor DNA binding sites transfected into the HepG2 human liver hepatoma cell line as previously described[9]. A major difference between the *cis* and *trans* system is that in *cis* activities of endogenous transcription factors are measured. This particular assay evaluated transcription for the Estrogen receptor element (ERE), and uses the code ATG_ERE_CIS. For this analysis, there were either 4 replicates in 16 concentrations, except for SAF-RON GOLD which was only tested in 2 replicates and 8 concentrations. Additional detail of the method is provided in the **Appendix B.1**. Concentration-response titration points for each compound were fitted as described in **Appendix C**.

Results: No statistically significant activity was seen for any of the dispersants

ER Agonist Assay 3 - ER-alpha beta-lactamase Assay

Method Summary: This assay was run at the NIH Chemical Genomics Center (NCGC; Rockville, MD) in collaboration with EPA as part of the Tox21 collaboration[10]. A beta-lactamase reporter-gene cell-based assay [ER α -UAS-*bla* GripTite™ cell-Based Assay from Invitrogen] was used to measure ER α signaling pathway both in agonist and antagonist modes. ER α -UAS-*bla*-GripTite™ HEK 293 cells (ER α *bla* cells) were used with assay medium containing 2% charcoal/dextran treated FBS, 0.1 mM NEAA and 1 mM sodium pyruvate. Cells were cultured in this assay medium overnight in the flasks before the assay. The assay was performed in clear bottom black Greiner 1536-well plates. 17 β -estradiol was used as a positive control in the screen. Library compounds were measured for their ability to either stimulate or inhibit the reporter gene activity. Compounds were screened in a titration series in 1536-well format. The fluorescence intensity (405 nm excitation, 460/530 nm emission) was measured using an EnVision plate reader. Data was normalized relative to 17 β -estradiol control (20 nM, 100%, for agonist mode and 0.5nM, 0%, for antagonist mode), and DMSO only wells (basal, 0% for agonist mode and -100% for antagonist mode). Concentration-response titration points for

each compound were fitted to the Hill equation yielding concentrations of half-maximal stimulation (EC_{50}), half-maximal inhibition (IC_{50}) and maximal response (efficacy) values. For this analysis, there were 8-10 replicates in 24 concentrations. Additional detail of the method is provided in the **Appendix B.3**. Concentration-response titration points for each compound were fitted as described in **Appendix C**.

Results: No biologically relevant results were seen for any of the dispersants. See the description above under the corresponding AR assay for JD 2000.

ER Agonist Assay 4 - T47D-KBluc estrogen-responsive transcriptional activation assay

Method Summary: T47D-KBluc, is an estrogen receptor-mediated transcriptional activation assay (ER-TA) that detects the ability of chemicals to mimic estrogen[8]. This assay was run in-house by NHEERL researchers. The cells contain endogenous human estrogen receptors alpha and beta and are stably integrated with an engineered luciferase reporter gene controlled by triplet estrogen response elements. When the cells are exposed to hormone mimics, the mimicking chemical binds the estrogen receptor and activates production of the luciferase reporter gene. The luciferase product is measured in a light emitting reaction. Additional detail of the method is provided in the **Appendix F**.

Results: The ability of the eight dispersants to stimulate luciferase expression in this cell line was compared to 17β -Estradiol (CAS 50-28-2: a concentration-response curve to E2 was included on each 96 well plate with the dispersants) and to 4-Nonylphenol (branched) (CAS 84852-15-3) (**Figure 7 a,b**). 17α -Trenbolone (CAS 80657-17-6) was run as a negative control herein (**Figure 7d**) and as a positive control in the assessment of androgenicity. None of the eight dispersants displayed any potential estrogenicity (i.e. did not simulate luciferase induction) at any concentration in the current investigation (**Figure 8**). In fact, all the dispersants significantly reduced luciferase levels at high concentrations due to cytotoxicity.

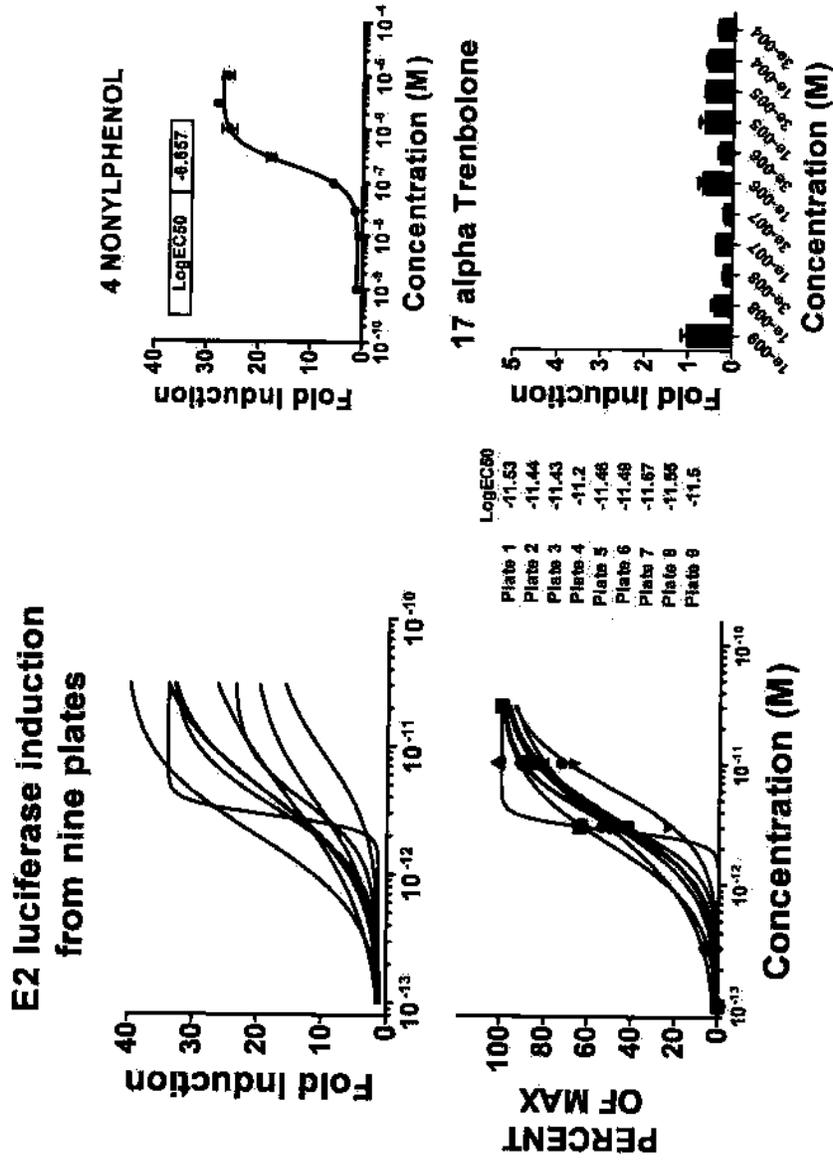


Figure 7: Estradiol 17β-induced (E2) luciferase expression in the nine plates used in the current study with T47D Kbluc cells, expressed as fold over media plus ethanol vehicle control (upper left) and percent of the maximal E2 stimulation (lower left). The

effects of the xenoestrogen 4-Nonylphenol (Branched) are shown in the upper right and the lack of estrogenicity of the synthetic androgen 17 α -Trenbolone are shown in the lower right panels. Values are means plus or minus standard errors of the mean.

Dispersants do not display any indication of estrogenic activity in T47D Kbluc cells

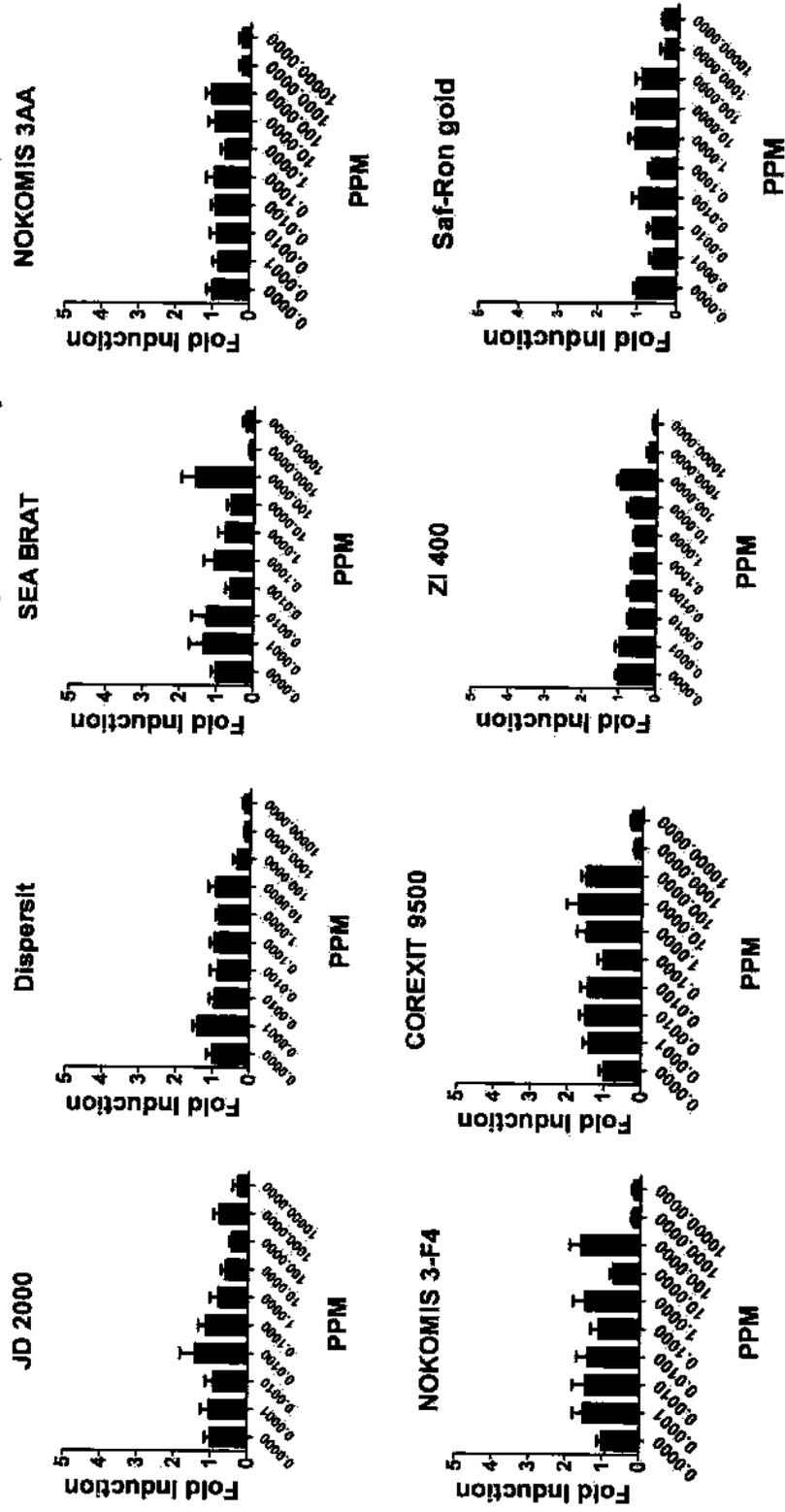


Figure 8: Assessment of the potential estrogenic activity of the eight dispersants in T47D Kbluc cells. Data are expressed as fold over the media plus ethanol vehicle control. Values are means plus or minus standard errors of the mean. Dispersants did not stimulate luciferase induction over the control fold value (control fold = 1).

Cytotoxicity

Cytotoxicity Assay 1 -HepG2 Cells

Method Summary: Dispersants were tested for cytotoxicity against HepG2 cells in the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium assay (15) following 24 h chemical exposure to 16 concentrations with an upper concentration of 1000 ppm. All concentrations were run in triplicate. This assay was run by Attagene Inc. LC50 values were determined by fitting curves as described in **Appendix C**. Results of cytotoxicity assessment are shown below. For this analysis, there were either 4 replicates in 16 concentrations, except for SAF-RON GOLD which was only tested in 2 replicates and 8 concentrations.

Cytotoxicity Assay 2 -AR *bla* Cells

Method Summary: Cell viability after compound treatment was measured in these AR *bla* cells using a luciferase-coupled ATP quantitation assay (CellTiter-Glo viability assay, Promega). This assay was run by the NIH Chemical Genomics Center. The change of intracellular ATP content indicates the number of metabolically competent cells after compound treatment. The cells were dispensed at 2,000 cells/5 μ L/well for AR *bla* cells in 1,536-well white/solid bottom assay plates using an FRD. The cells were incubated for 5 hrs at 37°C, followed by the addition of compounds using the pin tool. The final concentration range for reference compounds was 11 pM to 92 μ M, and 0.000144 ppm to 1209.8 ppm for dispersants. The assay plates were incubated for 16 hrs at 37°C, followed by the addition of 5 μ L/well of CellTiter-Glo reagent. After 30 min incubation at room temperature, the luminescence intensity of the plates was measured using a ViewLux plate reader (PerkinElmer). Data was normalized relative to DMSO only wells (0%), and tetra-n-octylammonium bromide (92 μ M, -100%). LC50 values were determined by fitting curves as described in **Appendix C**. Results of cytotoxicity assessment are shown below. For this analysis, there were 8-10 replicates in 24 concentrations.

Cytotoxicity Assay 3 -ER *bla* Cells

Method Summary: Cell viability after compound treatment was measured in these ER *bla* cells using a luciferase-coupled ATP quantitation assay (CellTiter-Glo viability assay, Promega). This assay was run by the NIH Chemical Genomics Center. The change of intracellular ATP content indicates the number of metabolically competent cells after compound treatment. The cells were dispensed at 5,000 cells/5 μ L/well for ER α *bla* cells in 1,536-well white/solid bottom assay plates using an FRD. The cells were incubated a 5 h at 37°C, followed by the addition of compounds using the pin tool. The final concentration range for reference compounds was 11 pM to 92 μ M, and 0.000144 ppm to 1209.8 ppm for dispersants. The assay plates were incubated for 18 hrs at 37°C, followed by the addition of 5 μ L/well of CellTiter-Glo reagent. After 30 min incubation at room temperature, the luminescence intensity of the plates was measured using a ViewLux plate reader (PerkinElmer). Data was normalized relative to DMSO only wells (0%), and tetra-n-octylammonium bromide (92 μ M, -100%). LC50 values were determined by fitting curves as described in **Appendix C**. Results of cytotoxicity assessment are shown below. For this analysis, there were 8-10 replicates in 24 concentrations.

Cytotoxicity Results (Assays 1-3)

Results of Cytotoxicity Assays 1-3 are summarized below the description of Assays 4-6.

Cytotoxicity Assays 4 THRU 9: measurements in T47D-KBluc, MDA-kb2, and CV-1 cells (MTT and CPE assessments) (5 independent assessments).

Methods summary: The ability of the dispersants to produce a general toxic effect on each of the cell lines used in the in the NHHERL in-house assays was assessed by both observational and biochemical methods. First, each well of cells in every assay was evaluated by visual microscopic examination utilizing a five point cytopathic effect (CPE) criteria scale ranging from 0 (no visual toxicity) to 4 (total cell death). CPE assessment criteria were as follows: 0 = no observed effect; 1= subtle changes suggesting effect; 2 = definite effects or death in a at least 25% of cells; 3 = 50 to 75% of cells effected; 4 = 100% of cells effected/cell death.

Second, an assessment of the metabolic perturbation of cell health was quantitated by monitoring the ability of cells to metabolize 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT)[11]. In this biochemical assay, healthy cells are capable of converting a yellow MTT solution into a blue dye. The healthier the cell the more blue dye produced. This biochemical assay is an indicator that the cells are metabolically active and is a measurement of general cell health. The MTT assay is a quantitative evaluation of mitochondrial function of the cells whereas the first method was a qualitative microscopic cytopathological evaluation (CPE) of cell viability and morphology

Cytotoxicity Results (Assays 4-9)

All eight dispersants disrupted cell function and caused cell death in all three cell lines in the two highest concentrations (0.01 and 0.001, or 10,000 and 1,000 ppm, respectively). Furthermore, none of the dispersants produced any sign of cytotoxicity at concentrations below 1 ppm (Figures 9-12).

June 30, 2010

Cytopathological evaluation of dispersant cytotoxicity (eight plates/dispersant, four replicate wells/plate/conc) in MDAKb2, CV 1 and T47D Kbluc cells. Figures are ranked from top left to bottom right in cytotoxic potency at 1, 10 and 100 ppm. At higher concentrations CPE scores were 3-4 for all dispersants and no CPE was observed at concentrations below 1 ppm. The maximum total CPE score is 12, 4 per assay

cytopathic effect: defined
 no CPE none observed
 CPE +1 subtle changes suggesting effect
 CPE +2 definite changes in more than 25 % of cells
 CPE +3 50-75 % of cells effected
 CPE +4 100% cells effected/dead

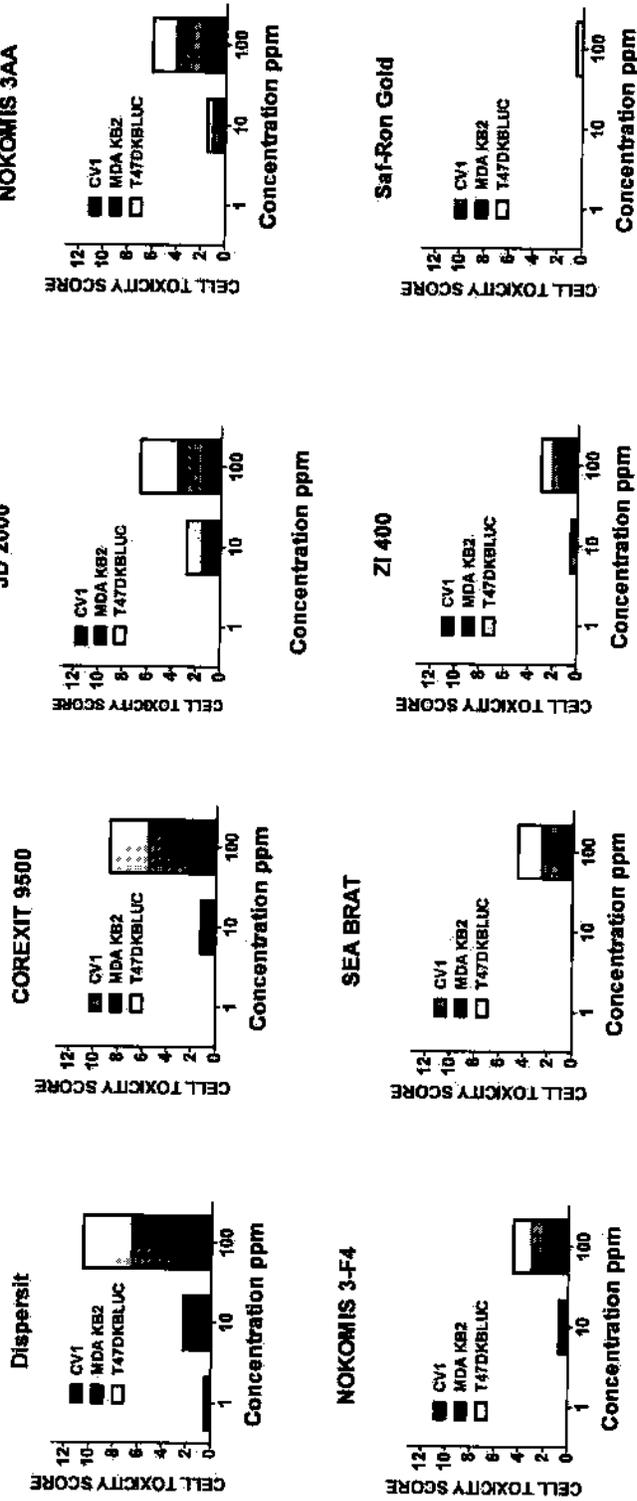


Figure 9:

MDA Kb2 CELL MTT ASSAY OF CELL FUNCTION

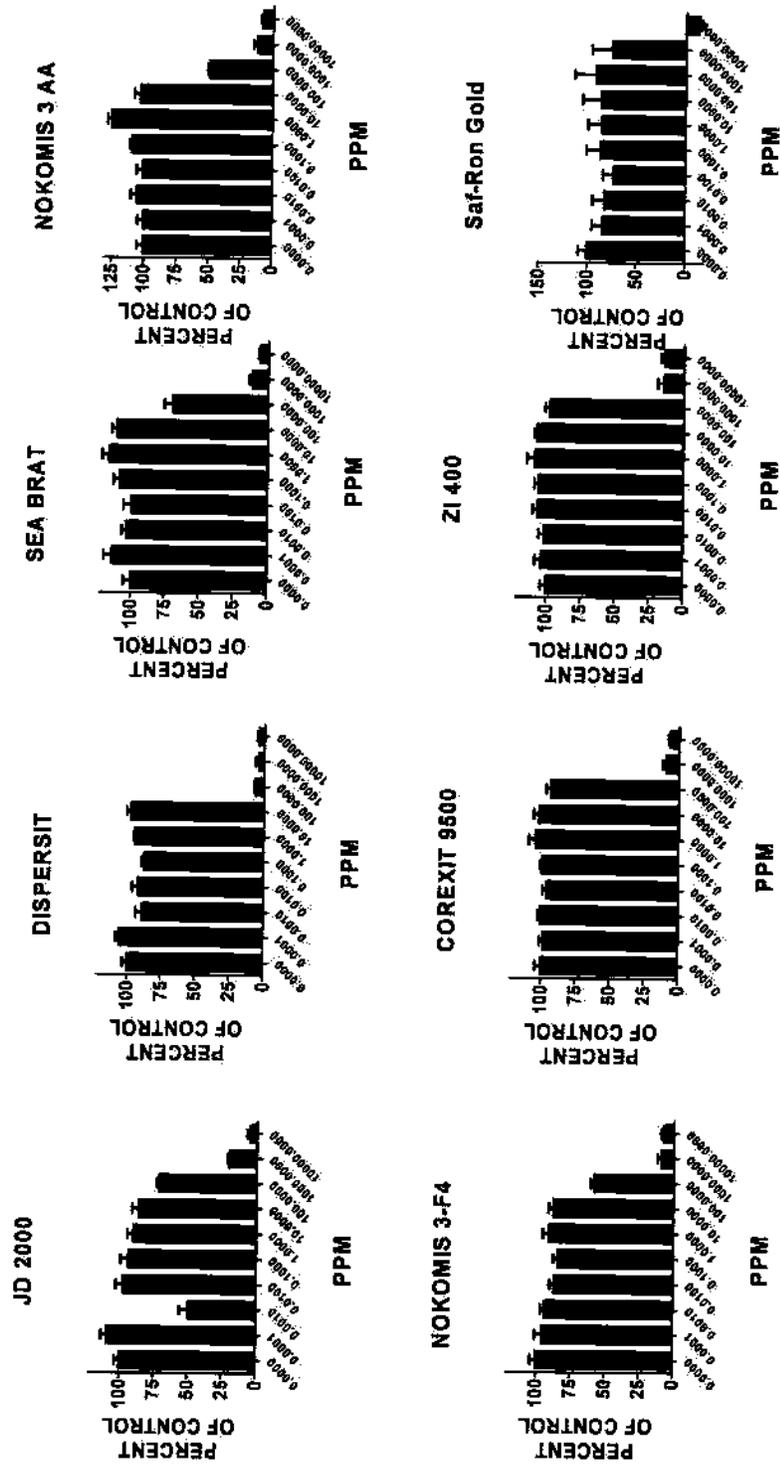


Figure 10: Toxic effects of the eight dispersants on MTT levels in MDA Kb2 cells. A reduction in MTT levels is an indicator of cytotoxicity, seen with all dispersants at the two higher concentrations (1000 and 10,000 ppm) and at 100 ppm with several of the dispersants. Data are expressed as percent of control (media).

CYTOTOXICITY OF DISPERSANTS TO CV-1 CELLS IN THE MTT ASSAY

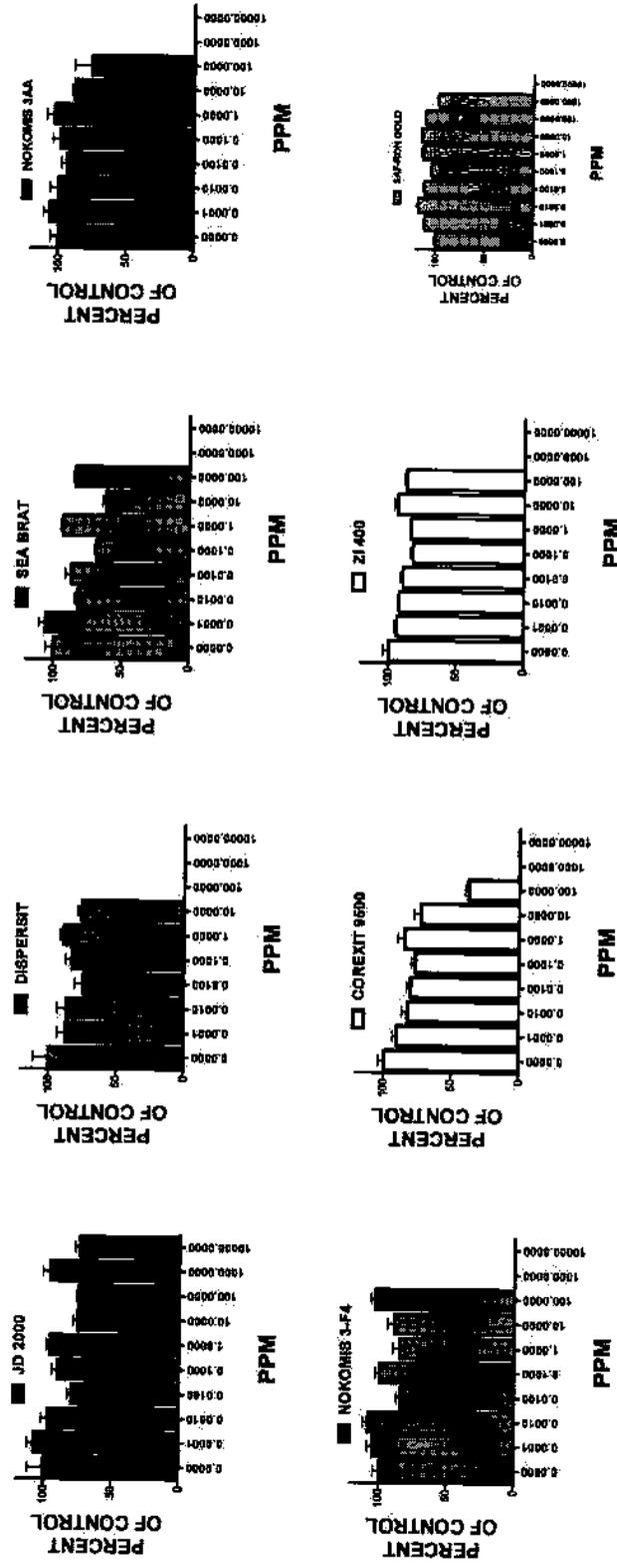


Figure 11: Toxic effects of the eight dispersants on MMT levels in CV-1 cells. A reduction in MTT levels is an indicator of cytotoxicity, seen with all dispersants at the two higher concentrations (1000 and 10,000 ppm) except JD 2000 (no toxicity was seen at any concentration) and SAF-RON GOLD (toxicity was seen only at the highest concentration of 10,000 ppm). DISPERSIT SPC 1000 and Corexit also induced cytotoxicity at 100. Data are expressed as percent of control (media).

Cytotoxic effects of dispersants on T47D Kbluc cells in the MTT Assay

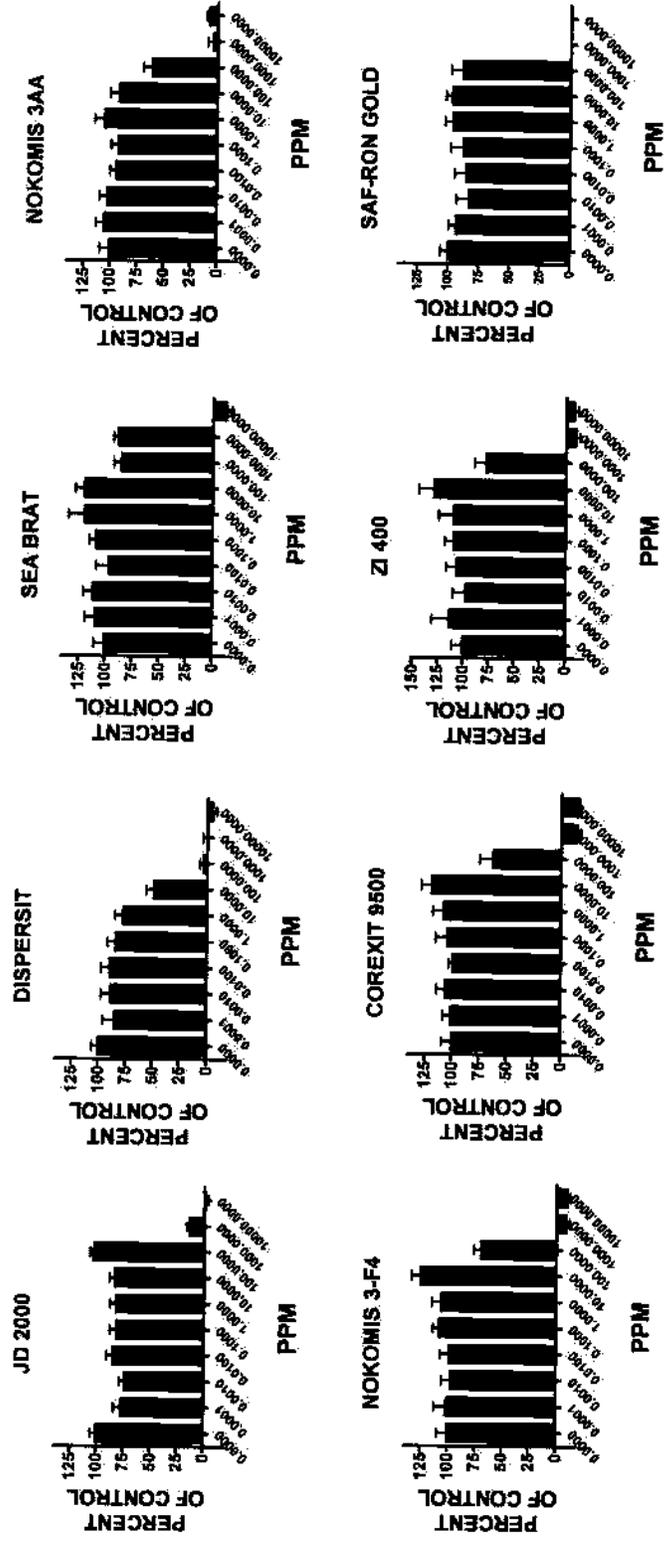


Figure 12: Toxic effects of the eight dispersants on T47D Kbluc cells. A reduction in MTT levels is an indicator of cytotoxicity, seen with all dispersants at the two higher concentrations (1000 and 10,000 ppm) and at 100 ppm with several of the dispersants. Data are expressed as percent of control (media).

The lowest observed effective concentration (LOEC) for dispersant-induced reductions in MTT, estrogen, androgen and antiandrogen assays are reported in Table 3. In the table, the noted changes in the two androgen and the estrogen agonist assays do not result from hormone-like increases in luciferase activity but rather represent significant reductions in luciferase expression that likely result from the cytotoxic effects of the dispersants. Statistical significance was determined using analysis of variance followed by t-tests (LSMEANS) using PROC GLM on SAS 9.1 ($p < 0.01$ was used as the critical value to determine statistical significance).

Dispersant	MDA Kb2 cells			CV1 cells		T47D Kbluc cells	
	MTT Cytotoxicity	Androgen Antagonist Assay***	Androgen Agonist Assay	MTT Cytotoxicity	Androgen Agonist Assay	MTT Cytotoxicity	Estrogen Agonist Assay
	LOEC* (ppm)	LOEC (ppm)	LOEC (ppm)	LOEC (ppm)	LOEC (ppm)	LOEC (ppm)	LOEC (ppm)
JD 2000	100	>10	1,000	10,000**	1,000	1,000**	10,000**
DISPERSIT SPC 1000	100	10	100	10	100	10	100
Sea Brat #4	100	>10	100	1,000**	1,000	10,000	1,000
Nokomis 3-AA	100	10	10	1,000	1,000	100	1,000
Nokomis 3-F4	100	>10	10	1,000	1,000	1,000	1,000
Corexit 9500	1,000	>10	1,000	100	1,000	100	1,000
ZI-400	1,000	>10	100	1,000	1,000	1,000	1,000
SAF-RON GOLD	10,000	>10	1,000	10,000	1,000	1,000	10,000

*LOEC (ppm) represents the lowest concentration at which the dispersant consistently reduced the MTT value. Statistical significance was using $p < 0.01$ as determined using LSMEANS option of PROC GLM available on SAS 9.1.
 ** LOEC concentration was equivocal (nonmonotonic response)
 *** Antagonist assay for antiandrogens was not run the three highest concentrations (10,000; 1000 and 100 ppm) to avoid most the confounding effects of cell death. The highest concentration was 10 ppm, Dispersants that did not reduce luciferase expression in this assay at any concentration were scored as >10 ppm.

Table 3: Summary table of the Lowest Observed Effect Concentration (LOEC) of the eight dispersants in the MTT cytotoxicity in three cell lines, the estrogen agonist assay in T47D Kbluc cells, the agonist assays in CV-1 and MDA K2 cells and the antagonist assay in MDA Kb2 cells. Since none of the dispersants displayed any effect interpreted as result of the dispersant displaying

endocrine activity we interpret all the results as indications of disruption of cell function and cell death. Since the androgen antagonist assay for antiandrogens did not include the three dispersant highest concentrations (10,000, 1000 and 100 ppm) to avoid most the confounding effects of overt toxicity (seen in the MTT assay with MDA Kb2 cells), the highest concentration in this assay was 10 ppm. Dispersants that did not reduce luciferase expression in this assay at any concentration were scored as >10 ppm. In spite of this precaution, the two most cytotoxic dispersants still reduced luciferase expression in this assay, an effect we attribute to less overt cell toxicity.

The EC₅₀ values for the dispersant dose response curves were determined using nonlinear regression procedures with GraphPad Prism 5.0 software (Figure 5 a,b,c). Ranking the eight dispersants in order of highest to lowest potency in the MTT assays and the CPE assessment in three cells lines indicates that there are some consistent differences among dispersants in their ability to disrupt the function and viability of these cell lines (Figure 5 d). Dispersant SPC 1000 appears to be more toxic in both MTT (below) and CPE assessments.

Cytotoxicity Summary

For comparison to all of the *in vitro* cytotoxicity assays, we also include LC50 values from whole animal, aquatic species lethality assays for the mysid, *Americamysis bahia*, in a 48-hr static acute toxicity test and an inland silverside, *Menidia beryllina*, 96-hr static acute toxicity test [12]. All LC50 values are plotted in Figure 13 and the numerical values are listed in Appendix D. One can see that the cell-based LC50 values overall vary by about two orders of magnitude, and that the values for any given chemical span about one order of magnitude. The rank order of cytotoxicity varied between the various cell types, a not unexpected finding [13]. There is overlap in the range of cytotoxicity for all of the dispersants.

In order to assess, statistically, differential cytotoxicity across the eight dispersants we performed an ANOVA to determine pairwise if any two dispersants were more cytotoxic than the other. We performed this statistical test with and without multiple test correction (Bonferroni). For any dispersant and assay combination that did not achieve an LC50, a default value of 3000 ppm was used; three-fold higher than the highest concentration tested in the relevant assays. LC50 values

greater than 3000 ppm were also set to this default value to prevent large extrapolated LC50 values from biasing the results. All six cell-based quantitative cytotoxicity assays were used for this analysis. The resulting p-values, raw and corrected, are provided in Table 4. Both JD 2000 and SAF-RON GOLD tend to be less cytotoxic than the other dispersants. Likewise, DISPERSIT SPC 1000 tends to be more cytotoxic than the other dispersants in the cell-based assays.

The aquatic species LC50 values are almost always lower than the cell-based LC50 values. As with the cell-based assays, JD 2000 is the least toxic in the whole animal assay.

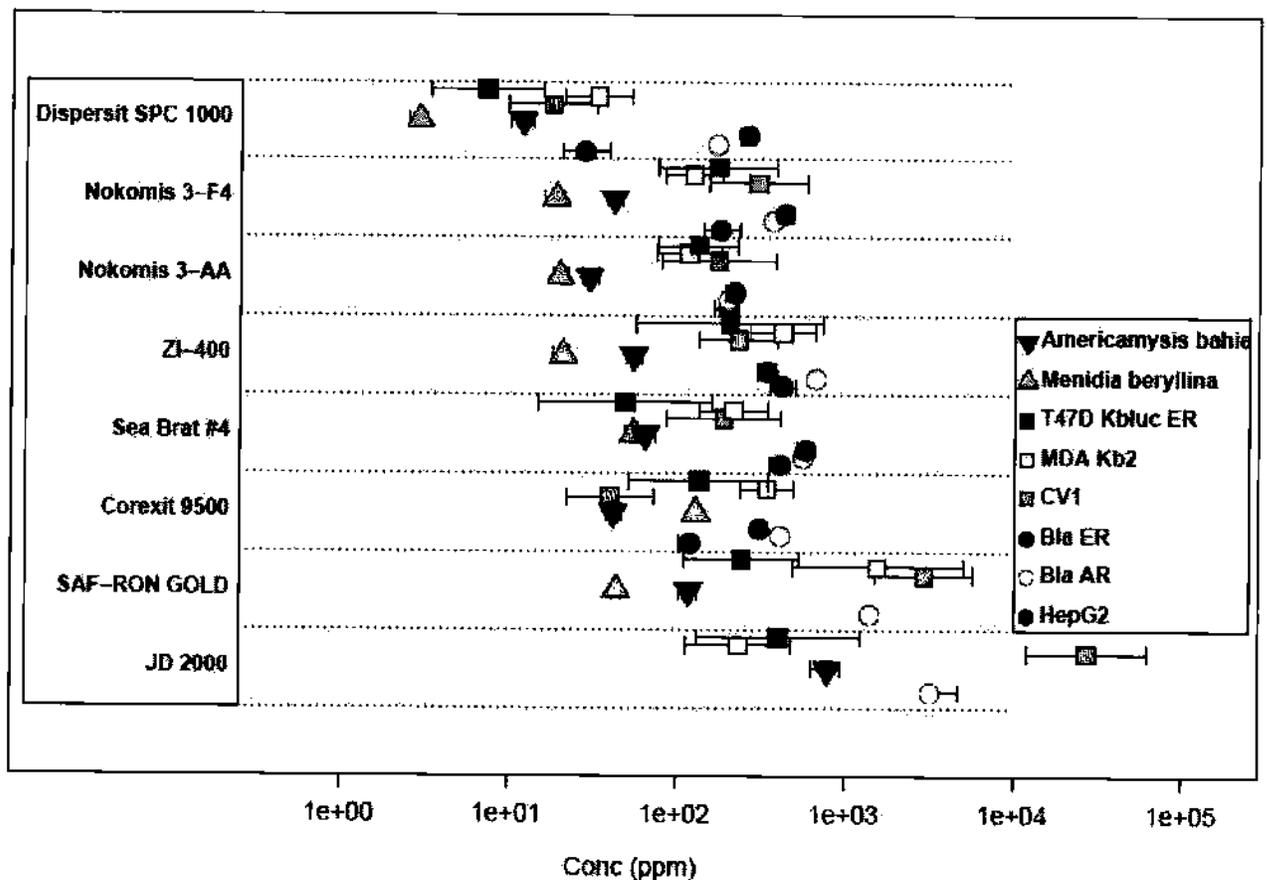


Figure 13: Toxicity data for the dispersants, combining data from cell-based assays in this report with data on aquatic species from a concurrent EPA report [12]. Each horizontal band shows the data for one dispersant. Results are presented from all 6 quantitative cytotoxicity assays. Cell-based LC50 values (concentration at which 50% lethality or effect is observed) are indicated by circles and

squares. Aquatic species LC50 values are indicated by triangles. Note that all dispersants were tested in all assays, and missing data points indicate that no toxicity was seen in that assay at the highest concentration tested. 95% confidence intervals are shown for all assays.

	JD 2000	Dispersit SPC 1000	Sea Brat 4	Nokomis 3-AA	Nokomis 3-F4	Corexit 9500	ZI-400	SAF-RON GOLD	Bonferroni Corrected P-Value
JD 2000		0.1456	0.308	0.1876	0.2464	0.224	0.364	1	
Dispersit SPC 1000	0.0052		0.84	1	0.644	1	0.126	0.056	
Sea Brat 4	0.011	0.03		1	1	1	1	0.1456	
Nokomis 3-AA	0.0067	0.082	0.11		1	1	0.42	0.0756	
Nokomis 3-F4	0.0088	0.023	0.5	0.12		1	1	0.1036	
Corexit 9500	0.008	0.086	0.34	0.42	0.65		1	0.0952	
ZI-400	0.013	0.0045	0.68	0.015	0.19	0.12		0.1652	
SAF-RON GOLD	0.92	0.002	0.0052	0.0027	0.0037	0.0034	0.0059		

Raw P-Value

Table 4: Statistical comparison of LC50 cytotoxicity values from cell-based assays across the eight dispersants. All dispersants combinations with a p-value less <0.05 are shaded pink. All values below the diagonal are raw p-values derived from the ANOVA, while all values above the diagonal were adjusted for multiple testing.

Other Molecular Targets

In addition to ER and AR, we also analyzed the chemical collection (dispersants plus reference chemicals) using a multiplexed reporter gene assay battery that evaluates activity against a panel of transcription factors including nuclear receptors[5, 9]. These assays were run by Attagene Inc. These data also provide a measure of quality control related to the specificity of any endocrine-related activity caused by the dispersants. The description of the assay and a complete list of targets is given in **Appendix B.2**. All of these assays were carried out twice, one week apart, and in each week, duplicate runs were performed. **Figure 14** summarize all of the results for the dispersants. This plot helps illustrate several key points about the data.

First, as the concentration of a chemical approaches the cytotoxic level, generalized cell stress occurs, accompanied by broad misregulation of transcription. When this threshold is reached, many assays in this system simultaneously activate, but this activity is assumed to be non-specific. One sentinel of this cell stress behavior is NRF2, which is an indicator of generalized oxidative stress. Therefore, if we see many assays become active at about the same concentration, especially if NRF2 is among them, we can discount any target specificity above that concentration. We see this behavior for Corexit 9500 (~50 ppm), JD 2000 (~500 ppm), Nokomis 3-AA (~75 ppm), Nokomis 3-F4 (~75 ppm), Sea Brat #4 (~90 ppm) and ZI-400 (~50 ppm).

The ER activity for Nokomis 3-F4 occurs at a concentration well below where this non-specific behavior is indicated. For ZI-400, the confidence intervals for ER and NRF2 overlap, indicating a possibility that the ER result is non-specific.

The lowest activity that is generally seen is for PXR (Pregnane-X-receptor), which is a xenosensor. This behavior is entirely expected, is common across many classes of organic chemicals, and is not in itself an indicator of toxicity. PXR has been reported to be a xenosensor that acts to protect against endocrine active chemicals[14]. PPAR (peroxisome proliferator activating receptor[15-19]) activity is observed for a number of the dispersants, at higher concentrations than is seen for the PXR assays. There is an extensive literature on PPAR activity

associated with disease in rodents, although the human relevance is unclear [15-18, 20-23]. However, only for Corexit 9500 and Nokomis 3-AA (and potentially for SAF-RON GOLD) is the PPAR signal well below the level of non-specific activity. Vitamin D receptor (VDR) activity is seen for Sea Brat #4 and Nokomis 3-AA below but near the concentration of non-specific behavior.

The activity of JD 2000 cannot necessarily be dismissed as being all non-specific, despite it occurring at the same concentration as NRF2 activity. This is because there are only two target families being activated – PXR and PPAR. A similar observation can be made about DISPERSIT SPC 1000. At the concentration of NRF2 activity, we only see activation of two PXR assays and one for SREBP (SREBF1 sterol regulatory element binding transcription factor 1) which is involved in fatty acid synthesis regulation.

The largest effect (in terms of EMax) of any dispersant and assays is for ZI-400 and AhR (Aryl hydrocarbon receptor), with EMax >30. The AhR is well-known for its role in mediating the adaptive metabolism of xenobiotics, and also in the toxicity that follows exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). This indicates the potential for the presence of a dioxin-like compound, which would be cause for concern. In the ToxCast Phase I data set[4, 5] of 309 chemicals, we saw only three with AhR efficacy higher than is seen with ZI-400. It is not clear though that this effect is specific, given that it occurs in the same concentration range as activity in a number of other targets, and above the NRF2 AC50.

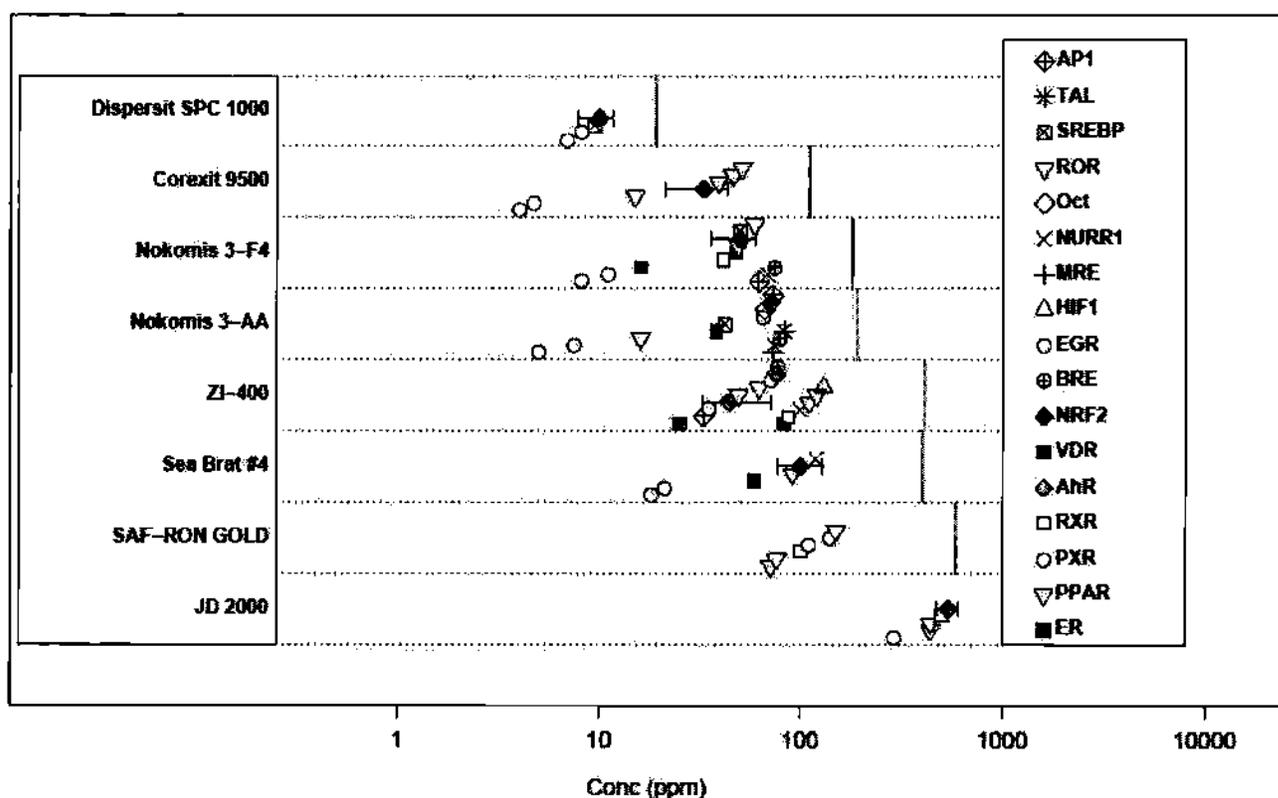


Figure 14: Summary plot of all Attagene *cis* and *trans* assays for dispersants with AC50 values below cytotoxicity levels. Each horizontal band displays data for a single dispersant. The x-value is the AC50. Points are staggered in the y-direction to make overlapping points visible. Where there were multiple assays for a given gene target (e.g. PPAR α , PPAR δ , PPAR γ) were given a single symbol. For illustration, 95% confidence intervals are shown on assays for NRF2. The vertical red lines indicate the LC50 for cytotoxicity in the HepG2 cells. Dispersants are ordered by decreasing cytotoxicity LC50 values.

The major conclusions of this section are that several of the dispersants display PXR and PPAR activity at concentrations below where cell stress and cytotoxicity occur. These are expected responses in hepatocytes to xenobiotics. The ordering of dispersants by lowest concentration at which bioactivity occurs is consistent with the ordering based on cytotoxicity. One observation of more general interest is that we are able to detect specific target-based bioactivity in complex mixtures such as these. This observation is relevant to the challenges of real world chemical toxicity testing, wherein humans and other organisms are often exposed to

complex mixtures rather than the pure single compounds that are the subject of typical toxicity testing.

Conclusions

The primary conclusions are as follows:

For six of the eight dispersants tested we found no evidence that they would be capable of interacting with estrogen or androgen receptor function from testing in multiple *in vitro* systems. For the other two dispersants, there was a weak ER signal in one assay. However, integrating over all of the ER and AR results, these data do not indicate that any of the eight dispersants will display biologically significant endocrine activity via the androgen or estrogen signaling pathways. As mentioned previously, NPEs (and their breakdown product NPs) can be endocrine disruptors in fish[1], so the risk of using NPE-containing dispersants should be carefully weighed against the expected benefits. One limitation of the present study is that there are other routes by which chemicals can cause endocrine disruption, as well as other types of toxicity that have not been tested for here. Most importantly though, there were no indications of estrogenic activity for Corexit 9500, the dispersant currently being used in the Gulf of Mexico.

All of the dispersants showed cytotoxicity in at least one cell type at concentrations between 10 and 1000 ppm. Both JD 2000 and SAF-RON GOLD tend to be less cytotoxic than the other dispersants. Likewise, DISPERSIT SPC 1000 tends to be more cytotoxic than the other dispersants in the cell-based assays. The aquatic species LC50 values tend to be lower than the cell-based LC50 values. As with the cell-based assays, JD 2000 is the least toxic in the whole animal assay.

Supplementary Information

Supplemental information, including a QA Statement, is included in the referenced Appendices.

QA Summary

All research described in this report was conducted under a comprehensive and rigorous program of quality assurance (QA), as documented in the QA supplemental file. The overall goal of the QA program was to ensure research data were of known and acceptable quality. QA staff surveillance of critical research activities was an important feature of the overall QA approach and ensured quick and effective resolution of any problems. The conclusion of the QA review process is that results presented in this report accurately reflect the raw data obtained during the course of the research and are scientifically valid and defensible.

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

MAY 26 2010

THE ADMINISTRATOR

Mr. David Rainey
Vice President of Gulf of Mexico Exploration
BP Exploration and Production
501 Westlake Park Boulevard
Houston, Texas 77079

Dear Mr. Rainey:

In your response dated May 23, 2010, you stated that you will continue to search for an alternative dispersant and that you fully understand and intend to comply with the directive from the U.S. Environmental Protection Agency and the U.S. Coast Guard to minimize the use of dispersants. I want to reinforce the importance of this approach to the BP oil spill response.

In the directive we sent last week, the EPA instructed you to analyze potential alternative dispersants for toxicity and effectiveness and report back within 24 hours. The goal of that directive was to determine whether a less toxic, more alternative dispersant existed in the quantities necessary to address this crisis.

Before I discuss the steps the EPA will take, I want to reiterate what Admiral Landry and I stated on a press conference call yesterday: The EPA and the Coast Guard believe your response to the directive was insufficient. We believe the response lacked sufficient analysis and focused more on defending your initial decisions than on analyzing possible better options.

Because we believe your analysis of potential alternative dispersants was insufficient, the EPA is performing its own scientific verification of the data BP presented. In addition, the EPA will perform testing to determine whether there is indeed a less toxic, more effective dispersant available in the volumes necessary for a crisis of this magnitude. The EPA will be performing at least two types of assessments to evaluate COREXIT 9500 and 9527 and other dispersants. Laboratory comparisons will be made with Gulf of Mexico species, including a silverside and Mysid shrimp. The EPA will use the quality assurance and testing methods set forth in the EPA test manuals (<http://www.epa.gov/waterscience/methods/wet/disk2/index.html>).

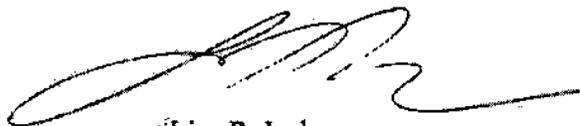
The EPA also will identify a test for endocrine disrupters and will use the test results to help make a determination in selecting less toxic dispersants.

Furthermore, as we discussed, the federal government, led by the Coast Guard, is reiterating its instructions to BP to take immediate steps to significantly scale back the overall use of dispersants. Data demonstrates that subsea dispersant application is having an effect on the oil at the source of the leak and thus far has had no observed significant ecological effects. Because so much is still unknown about the potential impact of dispersants, BP should use no more dispersant than is necessary. By decreasing the amount of dispersant used, particularly on the surface where we expect less undispersed oil because of the subsea application, BP can reduce the amount of dispersant applied by as much as 75 percent and possibly more.

Finally, I reiterate that BP must operate openly and transparently. Your response to EPA's directive contained redacted information because BP and dispersant manufacturers claim some sections of the response contain confidential business information. Once again, we demand that you immediately release to the public all of the information BP has claimed as CBI and urge you to do all you can to ensure Americans are fully informed about the potential environmental impact of these alternative dispersants.

I have attached a directive from the EPA and the Coast Guard requiring you to significantly reduce dispersant use. I fully expect your immediate and complete compliance with the requirements of the directive.

Sincerely,

A handwritten signature in black ink, appearing to read 'Lisa P. Jackson', with a long, sweeping horizontal line extending to the right.

Lisa P. Jackson

UNH Coastal Response Research Center, NOAA, EPA and Coast Guard Convene Science Meeting to Study Dispersant Use and Ecosystem Impacts of Dispersed Oil in the Gulf of Mexico.

Thursday, over 50 experts and practitioners from government, academia and industry finished a two-day meeting looking at the potential long-term impacts of the prolonged use of large volumes of dispersants in the Deepwater Horizon oil spill response efforts in the Gulf of Mexico. This is the third time NOAA and EPA have gathered top scientists to discuss dispersant use since the spill began. EPA and NOAA scientists are conducting rigorous ongoing monitoring and analysis of the effectiveness and toxicity of the dispersants used.

Should data indicate that the dispersants are causing significant environmental damage that outweighs the benefits of their use, EPA and the Coast Guard reserve the right to discontinue use.

Although the crude oil is more toxic than the authorized dispersants, much is unknown about the long term environmental impacts of dispersants when used in these unprecedented volumes on the surface and in the subsea. Because of this and due to the effectiveness of subsea applications, EPA and the U.S. Coast Guard directed BP to significantly ramp down their use of dispersants. BP has complied and has significantly reduced dispersant use.

The purpose of the two-day meeting was to provide input to the Gulf of Mexico Regional Response Teams (4 and 6) on the use of dispersants and the effects of dispersed oil going forward in the Deepwater Horizon incident. The meeting also identified possible monitoring protocols to be used in the event of continued aerial applications to surface water and subsea use.

"This conference provided us with additional scientific information about potential impacts of prolonged dispersant use that can help guide decision-making as we continue to support the U.S. Coast Guard's response to and clean up of this spill," said Craig Carroll, EPA Co-Chair of the Region 6 Regional Response Team.

"It is the consensus of the group that up to this point, use of dispersants and the effects of dispersing oil into the water column has generally been less environmentally harmful than allowing the oil to migrate on the surface into the sensitive wetlands and near shore coastal habitats," said Nancy Kinner, University of New Hampshire co-director of the Coastal Response Research Center.

"The meeting is adding to our knowledge, both in terms of helping identify key questions that should be asked and helping identifying new, quality sources of information and relevant expertise to draw on as we make these difficult decisions," said Charlie Henry, NOAA's Scientific Support Coordinator for the Unified Command Center in Roberts, La.

"The thoughtful scientific input from this meeting will prove valuable to responders as we continue to do everything possible to minimize damages caused by this unprecedented spill," said Robert Pond of the US Coast Guard.

This was the third science summit in three weeks that builds on the unprecedented mobilization of science the federal government has brought to this incident. The Administration has engaged some of the world's brightest scientific minds from the public and private sectors to mitigate the oil's impact and ensure an effective response.

The results of the meeting will be presented in a report to the Regional Response Teams within the next week. The report will be available on the CRRC website at www.crrc.unh.edu.

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**Dispersant Monitoring and Assessment Directive
for Subsurface Dispersant Application – Addendum 1
May 14, 2010**

This is an addendum (Addendum 1) to the Dispersant Monitoring and Assessment Directive issued on May 10, 2010, by the U.S. Coast Guard (USCG) and the Environmental Protection Agency (EPA) to BP. The requirements in this Addendum 1 apply to Part 2 of the May 10, 2010 Directive and are in addition to the requirements of that Directive. BP shall commence Part 2 requirements before subsurface application of dispersant is initiated and continue the Part 2 requirements and this Addendum 1 until cancelled or modified by the USCG and EPA.

Additional Requirements:

1. Sampling of dispersant/oil and oil-only waters must be continued per the Directive, and in addition, baseline data of waters without direct application of dispersant or oil shall also be collected by BP.
2. BP shall allow EPA/NOAA scientists flexibility within the sampling plan to direct the collection of additional data based on field observations (at times and locations of their choice). For example, EPA may request to recast the station if the CDOM fluorometer indicates a large increase in signal after data review. EPA/NOAA staff must be allowed to be in constant communication with staff on shore.
3. BP shall use Turner Designs C3 fluorometer (e.g., SMART protocol) to distinguish between oil impacted surface waters and those not impacted by oil.
4. BP shall use a CTD rosette package equipped with CDOM fluorometer and a 2-way communication wire to ensure that EPA/NOAA scientists can view profile data as the rosette package is deployed to 1500 meters. In addition, the CTD rosette package must be capable of collecting discrete samples in the water column using the live feed data stream. The requirement must be met within 7 days for the RV Brooks McCall. All other vessels must immediately meet this requirement.
5. BP shall deploy LISST from the vessel for continuous sampling of surface waters during transits, in order to provide particle size counts information which potentially distinguishes between dispersed and non-dispersed oil.
6. Discrete water samples shall be taken by BP at predetermined depths as specified or directed by EPA/NOAA scientists for UV fluorescences.
7. BP shall provide 48 hour advanced notice for departure and trip duration timelines to the FOSC and the EPA RRT Co-chair.
8. Data reporting shall be conducted by BP on a daily basis. This reporting shall include a sample tracking table. Data reporting shall be provided by BP to the FOSC and the EPA RRT Co-chair.

**Dispersant Monitoring and Assessment Directive
for Subsurface Dispersant Application
May 10, 2010**

Plume Monitoring and Assessment Plan for Subsurface Dispersant Application

BP shall implement the approved Dispersed Plume Characterization Plan for Subsurface Dispersant Application. Part 1 of the plan is a "Proof of Concept" to determine if subsurface dispersant operation is chemically dispersing the oil plume. Once the "Proof of Concept" test is complete, the results will be reviewed by the RRT for a decision to proceed or not proceed with Part 2 of the plan. Part 2 of the plan involves robust sampling to detect and delineate the dispersed plume. Part 3, entitled "Subsurface Injection of Dispersant", outlines the operational procedures. Additional guidance will be provided by the RRT coordination group on specific implementation of this directive and that guidance will be considered an addendum to this directive.

At least 24 hours prior to the testing, use and/or application of any subsurface dispersants, BP shall provide a *Dispersant Application Plan* that identifies the dispersants to be used, describes the methods and equipment used to inject the dispersant, plume model to assure representative sampling, proposed method of visual observation, process for determining the effectiveness of subsurface injection, the specific injection rate (i.e., gallons/minute), the total amount to be used for the duration of the test, the total length of time that dispersant is injected, and the plan for sampling and monitoring, as approved by the Unified Command Environmental Unit. Dispersants must be on the approved product schedule and suitable for this use.

All data shall be provided to the United States Coast Guard (USCG) Federal On-Scene Coordinator, and the Environmental Protection Agency (EPA) Regional Response Team (RRT) representative within 24 hours of the information being received. This data includes real time monitoring, laboratory analysis, documented observations, photographs, video, and any other information related to subsurface dispersant application.

BP shall conduct Part 1 monitoring and collect the data outlined below to determine dispersed plume concentration and transport. BP shall conduct Part 2 monitoring and collect the data outlined below, which will be sustained and more comprehensive, to address plume fate and effects on rotifers from the dispersed plume and chemical dispersants based on the results of Part 1 and iterative hydrodynamic modeling output.

Timing: BP shall commence Part 1 monitoring when subsurface application of dispersant is initiated. BP shall ensure that the R/V Brooks McCall or equivalent on location is outfitted, and manned before subsurface application commences.

Part 1

BP shall design and implement a Part 1 monitoring plan to determine the factors needed to calculate dispersion effectiveness, namely, % oil, % water, % dispersant. This phase of sampling should determine the factors to predict buoyancy; namely droplet sizes, density (or specific gravity) along the thermal gradient of the water column, and kinematic viscosity.

Part 2

If Part 1 is successful and continuous subsea injection proceeds, BP shall design and implement a Part 2 monitoring plan to collect and report, on a daily basis, the data and information described below. BP shall submit this plan to the FOSC and EPA RRT Co Chair for approval and shall begin implementation upon notice from the Coast Guard and EPA. BP shall continue implementation of this plan until further notification from the Coast Guard and EPA.¹

BP's monitoring plan shall include a more thorough oil analysis, to enable EPA to determine whether the dispersed plume is toxic to aquatic life. This plan shall be designed and implemented to determine whether the dispersed oil will hang in the water column and eventually come in contact with the benthos as it approaches land. BP has the option of conducting this particular monitoring and analysis as part of Part 1 if so desired.

PART 1 – Proof of Concept – Data Collection Requirement

- Towed Fluorometer at 1 meter
- LISST Particle Analysis at various intervals from surface to 550 meters
- Dissolved Oxygen at various intervals from surface to 550 meters
- CTD – Conductivity, Temperature, and Depth at various intervals from surface to 550 meters
- Water sampling from surface to 550 meters for PAH analysis
- Aerial Visual Observation (weather permitting)

PART 2 – Characterization Plan – Data Collection Requirement

- Cast Fluorometer – surface to sea floor
- LISST Particle Analysis at various intervals from surface to sea floor
- Dissolved Oxygen at various intervals from surface to sea floor
- CTD – Conductivity, Temperature, and Depth at various intervals from surface to sea floor
- Water sampling from surface to 550 meters for PAH analysis
- Aerial Visual Observation
- Rototox toxicity testing
- UV-Fluorescence testing to meet objectives in Appendix A

PART 3 – Subsurface Injection of Dispersant – Parameter Requirements

- Type of dispersant to be used
- Rate of dispersant injection

¹ See Appendix A for further background

- Process for monitoring pumping rate
- Procedures for FOSC to start and stop injection

Evaluation Criteria to Determine Operational Shut-Down of Subsurface Sea Dispersant Application:

The Federal On-Scene Coordinator will immediately convene the Regional Response Team (RRT) when either of the following conditions is reported:

1. If there is a significant reduction in DO from background to below 2 mg/L; or
2. For Part 2, if EPA's interpretation of the toxicity test reveals excessive exertion of a toxic response. To determine a measurable toxic response, BP must first perform a rangefinder test since the collection of the sample will be directly from the toxic plume, and any sample from the plume will likely kill 100% of the test population. Therefore, the rangefinder must first be conducted to determine an order of magnitude dilution that gives a measurable response. Then, a more refined dilution procedure must be done to get the final LC50 answer. This result will be compared to a NOAA plume model that would predict when or where exertion of that toxic response would take place. EPA and NOAA will interpret the results of the toxicity tests to inform determination of a shutdown decision.

The RRT will evaluate the conditions above, in addition to all relevant factors including shoreline, surface water, and other human health and ecological impacts, to determine whether subsurface dispersant application should be shut down.

Limitations to Address

BP shall include in its monitoring plan provisions to address and minimize the impact of the following challenges:

1. Timely transport of samples to labs where necessary, which may be subject to weather and/or operational delays.
2. Sampling in the deep sea environment may pose challenges due to equipment limitations and malfunctions.

Quality Assurance and Sampling Plan Requirements

BP's plan shall include sample collection methodology, handling, chain of custody and decontamination procedures to ensure the highest quality data will be collected. Discrete samples shall be tested at an approved lab(s). Duplicate samples shall be tested. All samples (or as practicably possible) shall be archived for potential future analysis. Where technically possible, all samples shall be at least 100 ml.

BP shall include the following components and criteria in its Sampling Plan:

1. An Introduction, to include project objective and project staff
2. A brief site description and background

3. A description of the Sampling Approach and Procedures, to encompass:
 - a. A brief overview of sampling activities, data quality objectives, and health and safety implementation strategies (frequently, this references another specific document, but must be included).
 - b. The actual sampling and/or monitoring approach, to ensure repeatability and consistent procedures. Describe sampling, monitoring, sampling and field QC procedures, spoil or waste disposal procedures resulting from this effort, as well as specimen/data handling issues.
 - c. Sample management – how the sample will be procured, handled, and delivered
 - d. Sample instructions- preservation, containers, and hold times
4. The analytical approach – what lab tests will be run, any special instructions, how the data will be verified, and how data will be reported.
5. Quality Assurance- custody procedures, field records including logs, chain of custody, qualitative data handling including photographs.

Special Monitoring of Applied Response Technologies (“SMART”) Protocol for Surface Application of Dispersants

BP shall immediately implement the Special Monitoring of Applied Response Technologies (“SMART”) Protocol (attached as Appendix B) at the Tier III level for surface application of dispersants. Results from Tier III monitoring must be shared with the Area Command Environmental Unit. If Tier III is not deemed to be sufficient, further direction will be provided.

Appendix A –Background for Part 1I Methodology for Informational Purposes

The fact that many organic compounds fluoresce at specific excitation and emission wavelengths is the basis for identifying many of the components of crude oil in seawater. When subject to excitation at 245-280 nm, polycyclic aromatic hydrocarbons (PAH) fluoresce over wavelengths of 310 to > 400 nm, depending on the number of aromatic rings in the structure. Only one group has examined the 2D UV Fluorescence Spectroscopy (UVFS) spectra of oil treated with chemical dispersants, the Ken Lee group at Fisheries and Oceans Canada (DFO). They found that a fixed excitation wavelength of 280 nm works best for fluorescence of PAHs in crude oil, and two different emission wavelengths, one at 340 nm for 1-and 2-ring PAHs and the other at 445 nm for 3-ring and higher PAHs, provide an excellent fingerprint for differentiating chemically dispersed oil from non-dispersed oil. As oil gets dispersed due to the action of a chemical dispersant, the peak height at 445 nm becomes highly pronounced relative to the peak height at 340 nm. Thus, computing the ratio of peak height at 340 to the peak height at 445 gives a direct measurement of the degree of dispersion that has taken place as a result of applying a dispersant to an oil.

The effect of oil dispersion on UVFS spectra can be expressed in terms of an emission ratio, so that dispersion can be tracked without having to measure oil concentration. The spectral changes associated with the application of dispersant can also be calibrated to quantify increasing oil or oil plus dispersant. The fact that UVFS and UVA data are comparable at an emission intensity of 445 nm or over the whole spectrum of intensities (from 300 - 500 nm) indicates that the fate of higher molecular weight (> 3-ring) PAH fractions - the more "dispersible" fraction of an oil slick - will provide a good idea of the fate of the oil as a whole during the dispersion process. Given that higher molecular weight PAHs may be associated with many of the persistent (or chronic) toxic effects of crude oils on marine organisms, the ability of UVFS to track "dispersible" fractions would make it a particularly useful tool in studies of the long-term toxic effects of dispersed oil.

Summary of EPA's Dispersant Monitoring and Assessment Directive for Subsurface Dispersant Application

Note: This monitoring and assessment plan for full-scale subsea application of dispersants will not be implemented until initial testing demonstrates the effectiveness of subsurface dispersant application.

Purpose: This directive requires BP to implement a monitoring and assessment plan for subsurface and surface applications of dispersants as part of the BP oil spill response. It also requires BP to include a more thorough oil analysis which will allow EPA to determine whether the plume is toxic to aquatic life.

The Plan is broken down into three parts:

Part 1: Determines if subsea dispersant operation is chemically dispersing the oil plume. To calculate dispersion effectiveness BP will collect data on the percent of oil, water and dispersant.

Part 2: Involves robust sampling to detect and delineate the dispersed plume. This sampling and monitoring plan will be a sustained and more comprehensive plan. It will address the fate of the plume and effects on rotifers from the dispersed plume and the dispersants.

Part 3: Outlines the operational procedures for subsurface injection of the dispersant and includes parameters such as the types of dispersant to be used, the rate of dispersant injection and how the pumping rate will be monitored.

Criteria to Shutdown Subsurface Dispersant Application:

This plan also defines the evaluation criteria for determining whether application of subsea dispersants should be shut down.

These criteria include:

- 1) a significant reduction of dissolved oxygen,
- 2) the results of rotifer toxicity tests and,
- 3) the evaluation of the conditions above in addition to other factors including shoreline, surface water, and other human health and ecological impacts.

Quality Assurance and Sampling Plan Requirements:

This monitoring and assessment plan requires that data collection management and analysis follows accepted standards to ensure that data is of the highest quality. Additionally, the plan outlines the criteria that must be included in all sampling and monitoring plans to ensure consistency and accuracy in the sampling process. All data will be given to the US Coast Guard and EPA within 24 hours of the information being received.

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IFR #72: Regarding dispersants, is Corexit more toxic than other alternatives that are available?

ANSWER: The product specified is more toxic than some products, but less toxic than others.

In accordance with 40 CFR 300 Subpart J, EPA approves dispersants for use in U.S. waters based on tests for toxicity and effectiveness. Any product listed on the schedule must meet a threshold minimum for effectiveness and test for, and report on, toxicity. No states have expressed reservations about the use of these dispersants in the past, as long as the dispersant is employed in accordance with the Regional Response Team Dispersant-Use pre-authorizations agreements established between the states and their federal partners at the regions around the country.

The toxicity data table at (http://www.epa.gov/emergencies/content/ncp/tox_tables.htm) provides toxicity data for the dispersants listed. Toxicity values should not be interpreted as absolute values, but rather, relative to one another in a general sense. For example, an LC50 of 4.49 should not be viewed as significantly different from an LC50 of 5.95. But, the LC50 of 4.49 can be viewed as significantly different from the LC50 of 42.00. Therefore, the toxicity values can be used to group dispersants (2 or 3 groups of similar toxicity), but should not be used to list dispersants according to toxicity (1 to 20).

All products on the National Contingency Plan Product Schedule are selected based on availability of volumes, specifics of the site, and the concerns of the Federal On-Scene Coordinator. Toxicity tests are methods for determining the impact of a chemical or an effluent on living organisms and measure the degree of response using commonly tested species. Many different kinds of tests can be used to identify potential toxic effects, but since toxic effects differ, comparing the toxicity of one to another may not be appropriate. In environmental studies, LC stands for "Lethal Concentration" and is the concentration of the chemical, given all at once, in the water that causes the death of 50% of a group of test animals in a given time (for example, during a 96-hour period). In general, the smaller the LC50 value, the more toxic the chemical. The opposite is also true: the larger the LC50 value, the lower the toxicity. For example, a chemical with an LC50 of 2 parts per million (ppm) would be more toxic than a chemical with an LC50 of 20 ppm. The LC50 is the measure of the immediate (or acute) toxicity of a chemical for the particular animal species being tested. The LC50 was not designed nor intended to give information on the long-term exposure effects of a chemical. It is also important to note that the LC50 value may be different for a given chemical depending on the route of exposure (e.g., skin contact, ingestion, inhalation) and can be different for different animal species, ages and sexes. The LC50 is only one source of toxicity information and only provides information for the species and concentrations of chemical being tested under laboratory conditions. Toxicity tests resulting from

controlled laboratory experiments may not accurately represent the degree of toxicity seen in the environment because of factors such as breakdown of the chemical, different species, different routes of exposure, age, sex, stage of development (e.g., adult versus larval).

Dispersant info at:

<http://www.epa.gov/bpspill/index.html>

**Statement by EPA Administrator Lisa P. Jackson from Press
Conference on Dispersant Use in the Gulf of Mexico with US
Coast Guard Rear Admiral Landry
May 24, 2010**

- Thank you for joining us. Let me take a moment to thank Admiral Landry for joining us today and for all the work she and all of our Coast Guard responders have been doing.
- They have shown extraordinary resolve in leading this effort. EPA is glad to be in partnership with them.
- Today we want to talk about three elements of our ongoing response and some of the adjustments we are making to this changing situation. But let me first outline what the situation is.
- The BP spill has thrust upon us what could potentially be one of the greatest environmental challenges of our time. More than 20,000 federal responders are continuing their work on creative solutions. Hundreds of EPA staff are focused on this crisis.

- In responding to this spill we have had to make some tough decisions – including the use of dispersant chemical to break up the oil and speed its natural degradation.
- Due to the unprecedented nature of this event, BP has used dispersants in ways never seen before. That is in terms of both the amount applied — which is approaching a world record — and in the method of application.
- A little more than a week ago EPA and the Coast Guard authorized, after testing for effectiveness, a novel use of dispersants underwater at the source of the leak.
- With that authorization, we required the implementation of a rigorous monitoring system, a condition that will ensure that underwater application continues to be effective and track any measurable environmental impacts.

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- Under the circumstances, the overall results to-date are positive. Our tracking indicates that the dispersants are breaking up the oil and speeding its bio degradation, with limited environmental impact at this time.
- In other words, dispersants continue to be the best of two very difficult choices. Their use inevitably means that we are making environmental trade-offs.
- But in all of this, it is critical to remember that the Number One enemy is the oil. Until we find a way to stem the flow of oil, we must continue to take any responsible action that will mitigate the impact of the spill. That is what we are doing.
- The steps we have taken are in full recognition of our tradeoffs.
 - We know that dispersants are less toxic than oil.
 - We know that surface use of dispersants decreases the risks to shorelines and organisms at the surface.

- And we know that dispersants breakdown over weeks rather than remaining for several years as untreated oil might.
- After testing and authorizing dispersant use underwater, we also remain optimistic that we are achieving similar results with the use of less chemicals.

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- We have put in place an extensive monitoring network to ensure the health of the air and water here. We have numerous stationary and mobile air monitors throughout the region – including a mobile unit that I personally inspected and toured today.
- To ensure the fullest level of transparency, all of the data we collect is being posted on www.epa.gov/bpspill as soon as we gather and analyze it.

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- We are still deeply concerned about the things we don't know. The long-term effects on aquatic life are still unknown and we must make sure that the dispersants that are used are as non-toxic as possible.
- Those unknowns – and the lengthening period of this crisis – are why we last week directed BP to look for more effective, less toxic alternative to their current dispersant. We felt it was important to ensure that all possible options were being explored, in the hopes that we might minimize the environmental tradeoffs in whatever ways possible.
- It's also why we have called on BP to be more transparent about their own processes. We have directed them to share information with the American people, who certainly deserve to know what actions we are taking.
- Which brings me to the three points we are here to discuss today.

- First, the federal government, led by the Coast Guard, is today instructing BP to take immediate steps to significantly scale back the overall use of dispersants.

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- Throughout this process, EPA and the Coast Guard have reserved the authority, in particular, to discontinue the use of underwater dispersants.
- As of today, our data demonstrates that subsea dispersant application is having an effect on the oil at the source of the leak – and thus far has had no significant ecological impact. That’s the good news. And we continue to monitor both whether the oil is being dispersed effectively and the impact of dispersant on the environment.
- But given our concerns over the environmental unknowns, we think it is prudent at this time to ramp down overall use of dispersants.

- This is possible because sub sea use appears to be having a positive effect. As a result, we should use no more dispersant than is necessary. By ramping down on the amount of dispersant used, particularly on the surface where we expect less un-dispersed oil because of the sub sea application, we believe we can reduce the amount of dispersant applied by as much as half, and possibly more.
- We will continue to track the effectiveness of this response. Admiral Landry of course reserves command control to decide if it makes sense to resume broader uses of dispersant.
- Second, we have made it clear to BP, including in a meeting Admiral Landry and I held with company officials last night, that we are not satisfied that BP done an extensive enough analysis of other dispersant options. We expect BP to keep evaluating other alternative dispersants.

- BP's response to our directive was insufficient, and we are concerned that BP seemed, in their response, more interested in defending their initial decisions than analyzing possible better options.
- So today we are calling on them to continue searching and studying better possible dispersant options.

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- Third, as a result of being dissatisfied with the response, and to ensure that we know everything we can know about the current environmental impact, EPA will be performing our own scientific verification of the data BP presented. We will conduct our own tests to determine the least toxic, most effective dispersant available in the volumes necessary for a crisis of this magnitude. Our toxicity tests will address the claims and conclusions put forth by BP in their response to us late last week. And EPA scientists have been tasked with conducting parallel, independent tests to determine if BP's argument that

Corexit remains the best alternative is accurate and supported by the science.

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- In the meantime, we will continue to do all we can to address this crisis in the most aggressive and responsible way possible. We will continue to aggressively monitor air quality, water quality and the effect of dispersants used by BP.
- This is unfortunately a tragic situation that presents a grave threat to the environmental, ecological and economic future of the Gulf region – a region I call home.
- The EPA and the entire federal government continue to work around-the-clock to do everything possible to ensure both that the citizens of the Gulf region are protected and that BP is putting every resource at their disposal toward stopping this leak.
- Thank you very much.

