

AN ABSTRACT OF THE DISSERTATION OF

Matt J. Perkins for the degree of Doctor of Philosophy in Toxicology presented on February 17, 2017

Title: Assessing Chemical Dispersants Used During the Deepwater Horizon Oil Spill:
Method Innovation and Application.

:

Abstract approved: _____

Jennifer A. Field

Large quantities of the chemical oil dispersant Corexit were applied in the Gulf of Mexico (Gulf) in response to the Deepwater Horizon oil spill. Large data gaps regarding the potential transport, persistence and impact of Corexit in the Gulf existed at the time of the emergency response. Analytical methods for the quantification of the individual surface-active-agent (surfactant) components of Corexit in seawater and sediments did not exist and needed to be developed for the support of environmental monitoring and laboratory experiments. The work presented in this thesis addresses important questions about the persistence and transport of the surfactant components of Corexit, namely DOSS, Span 80, Tween 80, and Tween 85 in marine systems. Unique challenges were uncovered and overcome along the way to answering these questions. Chapter 2 describes laboratory microcosm experiments quantifying the biodegradation of the Corexit surfactants in the presence and absence of oil using large volume injection liquid

chromatography tandem quadrupole mass spectrometry (LC-MS/MS). The extent of primary biodegradation differed for each surfactant and between treatments. Additionally, the microbial and abiotic hydrolysis of DOSS to its transformation intermediate EHSS was quantified. Significant biodegradation of DOSS was observed but mass balance with EHSS was not achieved, indicating that other transformation intermediates are formed during DOSS biodegradation. In contrast to a previous literature report, the abiotic hydrolysis of DOSS was not observed, suggesting that the abiotic losses observed by others were the result of the chemical biocide (sodium azide) employed in that study. Aqueous-phase surfactant concentrations were lower when Corexit was mixed with excess oil, due to partitioning of the surfactants into oil. The implication of this finding is that surfactant exposure concentrations depend on how Corexit is prepared. Chapter 3 describes the development and demonstration of an analytical method for the extraction and quantification of DOSS in marine sediments and sediment-trap materials using liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF). The use of QTOF resolved interferences without the need for sample cleanup that were observed in some samples analyzed by LC-MS/MS. Chapter 4 describes the application of the method developed in Chapter 3 to a large set of sediments collected in the Gulf between 2010 and 2015. Publicly available, but unpublished data, on DOSS in gulf sediments generated as part of the natural resource damage assessment (NRDA) were also incorporated and used to identify an area of DOSS-impacted sediments. The temporal trend in DOSS concentration from 2010-2015 indicates long-term persistence of DOSS with quantifiable DOSS concentrations remaining for more than 5 years after the application of Corexit during the Deepwater Horizon oil-spill. Chapter 5 describes the quantification of Corexit surfactants in aerosols generated under laboratory conditions that simulate bursting bubbles that occur under breaking waves. The ejection of the Corexit surfactants as aerosols was demonstrated. Collectively, the research presented in this thesis overcame analytical challenges in the measurement of the Corexit surfactants and informed the transport and persistence of DOSS and other surfactants in marine systems.

©Copyright by Matt J Perkins

February 17, 2017

All Rights Reserved

Assessing Chemical Dispersants Used During the Deepwater Horizon Oil Spill: Method
Innovation and Application

by

Matt J. Perkins

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Doctor of Philosophy

Presented February 17, 2017

Commencement June 2017

Doctor of Philosophy dissertation of Matt J. Perkins presented on February 17, 2017

APPROVED:

Major Professor, representing Toxicology

Head of the Department of Environmental and Molecular Toxicology

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Matt J. Perkins, Author

ACKNOWLEDGEMENTS

To Jennifer, thank you for the immense guidance, perspective, patience, and the resources you have given me. You have played an invaluable role in my life.

To Ruth, Dr. G, Renee, Glen, Herb and Erica, your passion for teaching and dedication to your students is a tremendous gift to the world and is reflected in the person I am today.

To mom and dad, thank you for everything. I love you.

CONTRIBUTION OF AUTHORS

Alix E Robel made significant contributions to the laboratory processing and extraction of sediments described in Chapter 4 and was instrumental to the success of the project.

Jennifer A. Field provided in depth guidance for the study design and resources for all of the work presented in this thesis. She also provided in depth guidance during the interpretation of data and the writing of the chapters presented in this thesis.

Samantha B Joye provided access to the microcosm samples presented in Chapter 2 and the sediment samples described in Chapters 3 and 4. She also contributed significantly to the interpretation of the data presented in Chapters 2 and 4. She also contributed to the writing of Chapters 3 and 4.

Sara Kleindienst carried out the microcosm experiments described in Chapter 2 at the University of Georgia.

Sairah Malkin created the additional Corexit-only and CEWAF solutions described in Chapter 2 at the University of Georgia.

Paria Avij conducted the bubble column experiments and the alkane analysis described in Chapter 5 at Louisiana State University. She also contributed significantly to the writing of Chapter 5.

He Huang contributed to sample collection and the processing of samples at Louisiana State University for the work described in Chapter 5.

Francisco R. Hung contributed to the writing of Chapter 5.

Kalliat T. Valsaraj is the Vice President for Research and Economic Development at Louisiana State University.

TABLE OF CONTENTS

	<u>Page</u>
CHAPTER 1: INTRODUCTION	1
1.1 Scope of Work	1
1.2 The Deepwater Horizon Oil Spill and the Decision to Use Dispersant in Response to DWH.....	1
1.3 Dispersant function	2
1.4 The Dispersant of Choice: Corexit	2
1.5 Use of Corexit during the Deepwater Horizon	3
1.6 Analytical Challenges Surrounding Corexit at the Time of the Spill	3
1.7 Artifacts of Experimental Design: The Challenges of Complex Surfactant Mixtures	4
1.8 Environmental Monitoring for Corexit Surfactants: The Challenges of Matrix Complexity.....	5
1.9 Literature Cited	7
CHAPTER 2 - COREXIT SURFACTANT BIODEGRADATION, HYDROLYSIS AND AQUEOUS PHASE COMPOSITION AFTER CEWAF FORMATION	12
2.1 Abstract	13
2.2 Introduction.....	14
2.3 Experimental	17
2.3.1 Chemicals and Standards.	17
2.3.2 Seawater Collection.	18
2.3.3 Corexit Only and CEWAF Solutions for Microcosms.	18
2.3.4 Microcosm Setup and Sampling.	19
2.3.5 Abiotic Controls.....	19

TABLE OF CONTENTS (Continued)

	<u>Page</u>
2.3.6 Additional Corexit-only and CEWAF ($\pm$ nutrients) solutions.....	19
2.3.7 Liquid Chromatography.....	20
2.3.8 Tandem Mass Spectrometry.	21
2.4 Results and Discussion	22
2.4.1 Degradation of the Corexit surfactants.	22
2.4.2 Abiotic hydrolysis of DOSS at 25° C.	27
2.4.3 Implications of Aqueous Phase Surfactant Composition.	24
2.5 Implications.....	28
2.6 Literature Cited	30
CHAPTER 3 - SELECTIVE QUANTIFICATION OF DOSS IN MARINE SEDIMENT AND SEDIMENT-TRAP SOLIDS BY LC-QTOF-MS	12
3.1 Abstract	34
3.2 Introduction.....	35
3.3 Experimental	36
3.3.1 Chemicals and Standards.	36
3.3.2 Sediment and Sediment-Trap Solids Collection.....	36
3.3.3 Sediment and Sediment-Trap Sample Preparation	37
3.3.4 Sediment and Sediment-Trap Solids Extraction.....	37
3.3.5 Liquid Chromatography	38
3.3.6 Triple Quadrupole Mass Spectrometry (LC-MS/MS).	38
3.3.7 High Resolution Quadrupole Time of Flight (QTOF) Mass Spectrometry.....	39
3.3.8 LC-QTOF-MS Calibration.	39

TABLE OF CONTENTS (Continued)

	<u>Page</u>
3.3.9 LC-QTOF-MS Quality Control.	40
3.3.10 Effects of Sample Processing and Storage.	40
3.3.11 LC-QTOF-MS Whole Method Accuracy and Precision.	41
3.3.12 Lower Limit of Quantification (LOQ).....	42
3.4.1 Extract Cleanup.....	42
3.4.2 LC-QTOF-MS Optimization.	43
3.4.3 Effects of sample processing and storage.	45
3.4.4 LC-QTOF-MS Method Demonstration.	45
3.4.5 Whole method evaluation of matrix effects.....	48
3.5. Conclusions.....	49
3.6 Literature Cited	50
CHAPTER 4 - SPATIAL AND TEMPORAL TRENDS IN DOSS IN GULF OF	
MEXICO DEEP-SEA SEDIMENTS: 2010 AND 2015.....	53
4.1 ABSTRACT.....	54
4.2 Introduction.....	55
4.3 Experimental	56
4.3.1 Chemicals and Standards.	56
4.3.2 Sediment Collection.....	56
4.3.4 Liquid Chromatography (LC) High Resolution Quadrupole Time of Flight (QTOF) Mass Spectrometry.	57
4.3.5 NRDA Dataset.	57
4.4 Results and Discussion.	57

TABLE OF CONTENTS (Continued)

	<u>Page</u>
4.4.1 Spatial Trend in DOSS Concentrations: 2010-2011.....	57
4.4.2 Temporal trends of DOSS impacted sediments 2010-2015.....	60
4.5 Implications.....	61
4.6 Literature Cited	62
CHAPTER 5 - EJECTION OF SURFACTANTS FROM COREXIT 9500A/9527A AND ALKANES FROM OIL INTO THE ATMOSPHERE VIA AEROSOLIZATION FROM THE SEA SURFACE	65
5.1 Abstract.	66
5.2 Introduction.....	67
5.3 Experimental	69
5.3.1 Materials and Standards.....	69
5.3.2 Laboratory Aerosolization Experiments.....	70
5.3.3 LC-MS/MS for Corexit Surfactant Analysis.	71
5.3.4 GC-MS and GC-FID for Alkane Analysis.	71
5.3.5 Calculation of Ejection Rates.	71
5.3.6 Salinity, Surface Activity, and Organic Carbon Content of Seawater.....	72
5.4 Results and Discussion.	72
5.4.1 The Ejection of Corexit Surfactants Detected by Nozzle Collection (NaCl Solution).....	72
5.4.2 The Ejection of Corexit Surfactants Detected by ESP Collection (NaCl Solution).....	76
5.4.3 The Effect of Seawater on the Ejection of Corexit Surfactants.	77
5.4.4 The Effect of Corexit on the Rate of Ejection of VOCs from NaCl Solution.	77

TABLE OF CONTENTS (Continued)

	<u>Page</u>
5.4.5 The Effect of Corexit on the Rate of Ejection of Volatile and Particulate IVOCs, SVOCs and NVOCs from NaCl Solution.	78
5.4.6 The effect of Corexit on the Rate of Ejection of Particulate IVOCs, SVOCs and NVOCs from NaCl Solution.	79
5.4.7 The Effect of Seawater on the Rates of Ejection of VOCs, IVOCs, SVOCs and NVOCs.....	80
5.5 Implications.....	81
5.6 Literature Cited	83
CHAPTER 6 – PARTING WORDS	89
6.1 The Big Picture.	89
6.2 Method Innovation, Application and Future Work.....	90
6.3 Literature Cited.	93
BIBLIOGRAPHY	95
APPENDICIES	104
Appendix 1 – Chapter 2 Supporting Information: Corexit Surfactant Biodegradation, Hydrolysis and Aqueous Phase Composition After CEWAF Formation	105
Appendix 2 – Chapter 3 Supporting Information: Selective Quantification of DOSS in Marine Sediment and Sediment-Trap Solids by LC-QTOF-MS	119
Appendix 3 – Chapter 4 Supporting Information: SPATIAL AND TEMPORAL TRENDS IN DOSS IN GULF OF MEXICO DEEP-SEA SEDIMENTS: 2010 AND 2015.....	123
Appendix 4 – Chapter 5 Supporting Information: EJECTION OF SURFACTANTS FROM COREXIT 9500A/9527A AND ALKANES FROM OIL INTO THE ATMOSPHERE VIA AEROSOLIZATION FROM THE SEA SURFACE	132

TABLE OF CONTENTS (Continued)

	<u>Page</u>
A4.2 Liturature Cited.	137

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
Figure 2.1. DOSS, EHSS, Span 80, and Tween 80 and Tween 85 in the dispersant-only and CEWAF ($\pm$ nutrients) treatments within 42 days of incubation.	23
Figure 2.2. (A) The surfactant composition of whole Corexit 9500 and the time zero mean surfactant composition of the Corexit-only and CEWAF ($\pm$ nutrients) treatments. (B) The composition of DOSS, EHSS and Tween 80 and Tween in whole Corexit 85 (i.e. Span 80 omitted) and additional Corexit-only and CEWAF initial solutions.	26
Figure 3.1. Interferences in the selected reaction monitoring chromatograms for the nominal-mass-to-charge transitions for DOSS (m/z 421 $\rightarrow$ 81 and m/z 421 $\rightarrow$ 227), $^{13}\text{C}_4$ -DOSS (m/z 425 $\rightarrow$ 81), and $^2\text{H}_{34}$ -DOSS (m/z 455 $\rightarrow$ 81) in a sediment and sediment-trap extract analyzed by LC-MS/MS	43
Figure 3.2. Typical extracted ion chromatograms of the molecular ions of DOSS (m/z 421.2265 $\pm$ 15 ppm), $^{13}\text{C}_4$ -DOSS (m/z 425.2400 $\pm$ 15 ppm), and $^2\text{H}_{34}$ -DOSS (m/z 455.4400 $\pm$ 15 ppm), and the confirmation fragment ion of DOSS (m/z 227.1278 $\pm$ 15 ppm) analyzed by LC-QTOF-MS	44
Figure 4.1. Spatial distribution of 39 sediment cores collected between 2010 to 2011 (white circles) and 2012 to 2015 (white circle with black dot), 18 sediment cores collected as part of the NRDA process on the Holiday Chouest cruise (+), and 114 sediment cores collected as part of NRDA on the Sarah Bordelon cruise (x).	56
Figure 4.2. DOSS sediment concentrations ($\mu\text{g kg}^{-1}$) collected by ECOGIG (green triangle), the Holiday Chouest (NRDA) cruise (red square), and Sarah Bordelon (NRDA) cruise blue diamond) in sediments plotted against radial distance from the DWH (km) in 2010 and 2011.	59

LIST OF TABLES

<u>Table</u>	<u>Page</u>
Table 3.1. Absolute and relative accuracy (average recovery $\pm$ 95% CI) and precision (% RSD) of spiked DOSS ($1.0 \mu\text{g kg}^{-1}$) and spiked $^{13}\text{C}_4$ -DOSS ($1.4 \mu\text{g kg}^{-1}$) onto a blank Gulf of Mexico sediment and the instrumental LOQ for DOSS determined from extraction blanks.	45
Table3. 2. Concentration of DOSS in extracts of marine sediment and sediment-trap solids, recovery of ^{13}C -DOSS, DOSS exact mass agreement (ppm), DOSS isotopic agreement (%), and latitude and longitude reported in degrees decimal minutes.	47
Table 4.1. ECOGIG data reported by year collected, DOSS concentration ($\mu\text{g kg}^{-1}$), radial distance from the DWH (km), latitude and longitude (decimal degrees), and the depth interval (cm) for surficial sediments observed to contain DOSS contamination.....	58
Table 5.1. Average rate of ejection ($\mu\text{g min}^{-1}$) of DOSS, Tween 80 and Tween 85 from NaCl solution and seawater.	74
Table 5.2. Normalized ejection rate ($\mu\text{g min}^{-1}$) (mean $\pm$ 95 CI) of VOCs (C10-C14), IVOCs (15-C19), SVOCs (C20-C24) and NVOCs (C25-C29) collected by nozzle and ESP.....	75

LIST OF APPENDIX FIGURES

<u>Figure</u>	<u>Page</u>
Figure A1.1. Chemical structure of DOSS (Bis-(2-ethylhexyl)sulfosuccinate) (A) and EHSS (α/β -ethylhexylsulfosuccinate) (B).	115
Figure A1.2. Chemical Structure and Tween 85 (A), Tween 80(B), and Span 80 (C)...	116
Figure A1.3. Corexit-only (A) and CEWAF (B) after 48 h of stirring.	117
Figure A1.4. Distribution of the primary surfactants in COREXIT and their expected distribution upon 1000X dilution (top panel) versus observed distribution in the Corexit-only solution (middle panel) and the CEWAF solution (lower panel).	118
Figure A2.1. Structure and molecular weight of DOSS (m/z 421.2265) and its characteristic fragment ions including (z)-4-((2ethylhexyl)oxy)4-oxobut-2-enoate (m/z 227.1289) and sulfonate (m/z 80.9165).....	122
Figure A4.1. (a) The actual picture of the bubble column reactor. (b) The schematic of the bubble column reactor adapted from Smith et al. and reprinted from Ehrenhauser et al.	136

LIST OF APPENDIX TABLES

<u>Table</u>	<u>Page</u>
Table A1.1. DOSS, EHSS, Span 80 and Tween 80 and Tween 85 concentration ($\mu\text{g L}^{-1}$) for triplicate microcosm samples at time zero and 7, 17, 28, and 42 days of incubation at 5°C	105
Table A1.2. DOSS, EHSS and Tween 80 and Tween 85 concentration ($\mu\text{g L}^{-1}$) for triplicate abiotic control microcosm samples prior to (pre) and after (post) double pasteurization and at 7 and 39 days of incubation at 8°C	109
Table A1.3. DOSS, EHSS and Tween 80 and Tween 85 concentration ($\mu\text{g L}^{-1}$) for triplicate abiotic control microcosm at time zero, 7 and 24 days of incubation at 25°C	114
Table A1.4. Mass (g) of DOSS, EHSS, Span 80 and Tween 80 and Tween 85 in 100 g of Corexit 9500 and the surfactant to EHSS ratio.	110
Table A1.5. The surfactant to EHSS ratio for DOSS, EHSS, Span 80, and Tween 80 and Tween 85. for triplicate Corexit –only, CEWAF and CEWAF + nutrients microcosm samples at time zero.	111
Table A1.6. The concentration of EHSS ($\mu\text{g L}^{-1}$) calculated using a bulk dilution factor, the measured EHSS concentration ($\mu\text{g L}^{-1}$), and the fraction of EHSS incorporated into the aqueous phase for the Corexit-only, CEWAF ($\pm$ nutrients) treatments, abiotic controls, and additional Corexit-only and CEWAF ($\pm$ nutrients) solutions.	112
Table A1.7. DOSS and EHSS concentration ($\mu\text{g L}^{-1}$) for additional triplicate t time zero Corexit-only and CEWAF ($\pm$ nutrients) initial solutions.	113
Table A2.1. DOSS concentration ($\mu\text{g kg}^{-1}$) calculated for extraction blanks.	120
Table A2.2. The effect of freeze drying and storage on DOSS concentrations . Mean DOSS concentration ($\mu\text{g kg}^{-1}$), and 95% confidence interval, and RSD calculated for replicate extracts of a single sediment sample containing environmentally-incurred DOSS	121
Table A3.1. Year collected, DOSS concentration ($\mu\text{g kg}^{-1}$), radial distance from the DWH (km), latitude and longitude (decimal degrees), and the depth interval (cm) for surficial sediments collected between 2010 and 2015 where DOSS was not overserved above the LLOQ ($0.23 \mu\text{g kg}^{-1}$).	126
Table A3.2. Year collected, DOSS concentration ($\mu\text{g kg}^{-1}$), radial distance from the DWH (km), latitude and longitude (decimal degrees), and the depth interval (cm) for surficial sediments collected on the Holiday Chouest cruise. LLOQ = $5 \mu\text{g kg}^{-1}$	127
Table A3.3. Year collected, DOSS concentration ($\mu\text{g kg}^{-1}$), radial distance from the DWH (km), latitude and longitude (decimal degrees), and the depth interval (cm) for surficial sediments collected by the NRDA program on the Sarah Bordelon cruise. LLOQ = $5 \mu\text{g kg}^{-1}$	128

LIST OF APPENDIX TABLES (Continued)

<u>Table</u>	<u>Page</u>
Table A4.1. Average ejection rate ($\mu\text{g min}^{-1}$) for VOCs (C10-C14), IVOCs (15-C19), SVOCs (C20-C24) and NVOCs (C25-C29) collected by nozzle and ESP.	135

**Assessing Chemical Dispersants Used During the Deepwater Horizon Oil Spill:
Method Innovation and Application**

CHAPTER 1: INTRODUCTION

1.1 Scope of Work

Analytical methods for the quantification of the surfactant components of chemical dispersant in seawater and marine sediment are challenged by the inherent amphiphilic nature of surfactants and the complexity of environmental sample matrices.³ However, determining the abundance and existence of surfactants in complex matrices is critical to toxicological studies,⁴⁻⁹ fate and transport assessments,^{1, 10-21} and potentially litigious investigations when used in response to oil spills.²² The following thesis describes several analytical challenges that were observed during the development of analytical methods to the surface-active-agents (surfactants) in oil dispersants in seawater and marine sediment and the application of these methods for the support of laboratory studies and environmental monitoring. Specifically, this thesis addresses the surfactant components of the chemical-oil-dispersant, Corexit, applied in the Gulf of Mexico (Gulf) in the aftermath of the Deepwater Horizon (DWH) Oil Spill.

1.2 The Deepwater Horizon Oil Spill and the Decision to Use Dispersant in Response to DWH

The unprecedented blowout of the oil well that was the DWH incident ultimately resulted in the largest accidental marine oil spill in international history.²³ Additionally, the spill occurred in the deep-sea, approximately 1500 m below the surface.²⁴ As a result, government, industry, and the scientific community were unprepared to respond quickly and effectively. Emergency responders were forced to make oil spill response decisions in the face of significant data gaps.²³ History, however, such as the Exxon Valdez, has left little doubt regarding the sensitivity of near-shore ecosystems to spilled oil.^{25, 26} Perhaps because of this response actions focused on protecting the Gulf coast ecosystems through the use of chemical oil dispersant.

1.3 Dispersant function

Chemicals including surfactants, which are used as dispersants during an oil spill are intended to remove oil from the sea surface, thereby reducing the degree of shoreline oiling that might otherwise occur.²⁷ The surfactant components of a chemical dispersant are key to increasing the volume of spilled oil that can be dispersed by turbulent wave action.²⁸ Surfactants are amphiphilic and as a result, when a chemical dispersant is delivered to a surface-oil slick, the surfactants associate at the oil-water interface and reduce the surface tension of the oil.^{27, 29} Sufficiently high dispersant to oil ratios (DOR) allow for the formation of smaller oil droplets than would form in the absence of a chemical dispersant (i.e. under physical dispersion alone).³⁰ This occurs for two reasons: 1) smaller droplets are more efficiently dispersed in the water column than larger droplets due to their lower buoyancy, and 2) lower energy conditions are required to produce small droplets.³¹

1.4 The Dispersant of Choice: Corexit

Corexit is the brand name of the commercial surfactant mixture that was applied as the chemical oil dispersant in the Gulf during the DWH emergency oil spill response action of 2010. In reality, two formulations of Corexit were applied: Corexit 9527A (C9527A) and Corexit 9500A (C9500A). During the initial stages of the DWH, the chemical composition of the proprietary Corexit formulations were unknown to the public.³² Since then, the general formulations have been released by the EPA and specific components have been quantified in the whole Corexit formulation prior to application in the environment.^{12, 32, 33} Both formulations are composed of a carrier solvent and surfactants. The major difference between C9527A and C9500A is the replacement of the carrier solvent 2-butoxy ethanol in C9527A with dipropylene glycol butyl ether in C9500A.²⁷ This substitution was made primarily to reduce toxicity.²⁷

The surfactant components of Corexit include the anionic surfactant bis-(2-ethylhexyl)sulfosuccinate (DOSS) and the nonionic surfactants Span 80, Tween 80 and Tween 85.²⁷ The nonionic surfactants are composed of complex mixtures of mono, di, tri,

and tetra substituted polyethylene oxide fatty acid ester and fatty alcohol ether derivatives of sorbitan. Further, each of these species is comprised of homologs that vary in the degree of ethoxylation.^{34, 35} The mass percent contribution of the surfactant constituents is similar between the two formulations of Corexit (DOSS, 10-22%; Span 80, 2.7-4.4%; Tween 80, 11-18%; and Tween 85, 4.3-4.6%).^{1, 11, 33} Impurities also exist in the whole formulation of Corexit (e.g. α/β -ethylhexylsulfosuccinate or EHSS, 0.28%).¹

1.5 Use of Corexit during the Deepwater Horizon

Over the course of the DWH emergency response, approximately 7 million liters of Corexit were applied to surface-oil slicks (3.9 million L) and directly at the well-head (2.1 million L). Both C9527A and C9500A were applied to surface slicks, while only C9500A was applied at depth.³⁶ The decision to apply Corexit directly at the well-head was made in an attempt to increase the dissolution of oil into the water column before it reached the surface, potentially limiting the volume of dispersant used and concurrently reducing the exposure of emergency responders to volatiles.²⁷ Both C9527A and C9500A were used previously in the Gulf in small volumes (<20 thousand L each application) in response to seven oil spills that occurred between 1998 and 2004.²⁷ However the use of Corexit during the DWH was unique for two reasons: this was the largest application of Corexit applied by several orders of magnitude and DWH was the first deep-sea application of a chemical oil dispersant.²³

1.6 Analytical Challenges Surrounding Corexit at the Time of the Spill

At the time of the DWH blowout at the well head, there existed significant data gaps in the persistence and toxicological impact of Corexit and chemically-dispersed oil in deep-sea and pelagic (i.e. open ocean) ecosystems.²² Similarly, the composition of Corexit was proprietary and, for this reason, analytical methods for the quantitative analysis of the individual components of Corexit did not exist for seawater and sediments.³² As the scientific community rushed to fill these data gaps, the experimental challenges of laboratory experiments often focused on creating environmentally-relevant conditions for hydrocarbons and did not include the individual components of Corexit.^{7, 8,}

³⁷⁻⁴² Quantitative analytical methods for the individual components of Corexit were developed for seawater but focused on the carrier solvents^{12, 13, 33} and DOSS.^{1, 10-14} Only a single study quantified the nonionic surfactants in a limited number of samples.¹ Furthermore, quantitative methods for sediments were not initially developed. This thesis presents collaborative experiments aimed at understanding the transport and persistence of the Corexit surfactants in environmental systems and solutions to experimental and analytical challenges with a focus on the Corexit surfactants in sediment.

1.7 Artifacts of Experimental Design: The Challenges of Complex Surfactant Mixtures

The study described in Chapter 2 was intended to provide further insight into the biodegradation of the surfactant components of Corexit that might occur in Gulf deep-waters. During the DWH, a prominent deep-water (~1100 m) hydrocarbon plume extended ~250 km south west of DWH.⁴³ The plume also contained DOSS that was observed to persist for ~6 weeks before being diluted below detection.¹⁰ Chapter 2 documents a collaborative laboratory microcosm experiment that mimicked a chemically-dispersed, deep water hydrocarbon plume using indigenous microbial communities collected in Gulf deep-water (~1100 m). This study addresses data gaps by quantifying the degradation of all the surfactant components of Corexit 9500A. Additionally, a high-purity standard of EHSS and its isotopically-labeled analog were synthesized at OSU⁴⁴ and used for the quantitative analysis of EHSS. EHSS has been reported as a microbial and abiotic hydrolysis product of DOSS.^{45, 46} In this research, EHSS was quantified to establish mass balance, or the lack thereof, for DOSS in laboratory microcosms.

The microcosm study also demonstrates the challenges of working with complex mixtures of surfactants. The specifics of the experimental design are described in detail in Chapter 2. Briefly, two Corexit solutions we prepared that contained either Corexit in seawater or a chemically-dispersed oil-Corexit mixture in seawater (chemically enhanced water accommodated fraction or CEWAF). Significant differences in the initial aqueous phase surfactant composition were observed between the dispersant-only and CEWAF solutions. A lower surfactant concentration in CEWAF occurred because more than 98%

of the surfactant components partitioned out of seawater and associated with the free oil phase and were discarded when the aqueous phase (i.e. CEWAF) were separated from the seawater phase. The change in surfactant concentration and composition due to partitioning to the free oil phase had not been reported in peer-reviewed literature. Previous reports on the chemical composition of CEWAFs focus only on changes to the hydrocarbon composition.

Chapter 2 also describes experiments undertaken to clarify claims of another group, Campo et al., specifically that DOSS undergoes rapid abiotic hydrolysis at 25° C.¹⁶ In contrast, our preliminary experiments indicated that DOSS did not hydrolyze easily, even under elevated temperature and pH (data not shown). We hypothesized that the abiotic hydrolysis observed by Campo et al. was not abiotic hydrolysis, but the result of adding a strong nucleophile (sodium azide) as a biocide. Therefore, additional experiments were undertaken to quantify the abiotic hydrolysis of DOSS over 28 days at 25° C.

1.8 Environmental Monitoring for Corexit Surfactants: The Challenges of Matrix Complexity

Chapter 3 builds off the observed partitioning of surfactants to free oil documented in Chapter 2. Approximately half of the 4.9 million barrels of oil spilled during DWH rose to the surface of the Gulf and 3.1 million L of Corexit were applied to surface waters of the Gulf.²² In the aftermath of the DWH, unusually large quantities of marine snow were observed and resulted, in part, from the presence of aging surface oil.⁴⁷ These massive marine snow events are now recognized to have played a major role in the sedimentation of DWH surface oil to the seafloor.⁴⁸ Therefore, DOSS could have been transported with surface oil to the sea floor.

At the time of this work, there were no established methods in the literature for the extraction of DOSS from marine solids (sediments or sediment trap materials). Chapter 3 describes the development of a methanolic extraction and quantification of

DOSS from marine solids using large volume injection liquid chromatography tandem mass spectrometry (LVI-LC-MS/MS). Complications were observed in the multiple reaction monitoring chromatograms for DOSS and its isotopes that compromised the analysis of some samples by LC-MS/MS. These mass spectral interferences were presumably caused by matrix components that partition very similarly to DOSS. Ultimately, the greater selectivity of quadrupole time of flight (QTOF) mass spectrometry sufficiently resolved these interferences without the need for further sample cleanup. Chapter 3 details the sample processing, storage stability, accuracy and precision of the of the LC-QTOF method. Additionally, a method demonstration is provided using a limited number of sediment and sediment-trap materials (i.e. time course samples of solids actively undergoing sedimentation) collected in the Gulf.

Chapter 4 then describes the application of the method presented in Chapter 3 to a larger number of sediment samples collected between 2010 and 2015. There were no literature reports of the presence of DOSS in deep-sea sediments at the outset of this work. However, a single study has been published reporting the presence of DOSS in a limited number ($n = 9$) of deep-sea sediments collected in 2010.⁴⁹ Chapter 4 presents the data for a larger number of sediments collected for OSU between 2010 and 2015. Furthermore, publically-available, but unpublished, DOSS concentrations in sediments that were reported as part of the National Resource Damage Assessment (NRDA) process are incorporated into Chapter 4 to generate the largest dataset on sediment DOSS concentrations reported in the literature. The spatial and temporal trends of DOSS concentrations in the Gulf sediments are discussed. Importantly, the long term persistence of DOSS in gulf sediments within 13 km of the DWH is documented with quantifiable concentrations for sediments collected in 2015.

A potential alternative fate of surface-applied DOSS is documented in Chapter 5: the ejection of surfactant aerosols by bursting bubbles. Aerosol production during turbulent wave conditions is known to contribute to particulate matter flux into the atmosphere.⁵⁰ Laboratory experiments simulating breaking wave conditions previously demonstrated the ejection of semi-volatile and nonvolatile alkane aerosols from seawater-crude oil mixtures by bursting bubbles.⁵¹ Chapter 5 describes laboratory experiments

undertaken to probe the potential for the ejection of surfactant aerosols from Corexit-oil mixtures by bubble bursting, such as that which may occur in the environment under turbulent conditions. Laboratory bubble column experiments conducted with crude oil mixed with Corexit 9500A or 9527A demonstrate for the first time that the surfactant constituents of Corexit (DOSS, Tween 80, Tween 85, and Span 80) are ejected as aerosols by bursting bubbles. Further, the presence of Corexit significantly increased the ejection of semi-volatile and nonvolatile alkanes relative to oil-seawater systems without Corexit.

1.9 Literature Cited

1. Place, B. J.; Perkins, M. J.; Sinclair, E.; Barsamian, A. L.; Blakemore, P. R.; Field, J. A., Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. *Deep Sea Research Part II: Topical Studies in Oceanography* **2014**.
2. Trufelli, H.; Palma, P.; Famiglini, G.; Cappiello, A., An overview of matrix effects in liquid chromatography–mass spectrometry. *Mass spectrometry reviews* **2011**, *30*, (3), 491-509.
3. Kaufmann, A.; Butcher, P.; Maden, K.; Widmer, M.; Giles, K.; Uría, D., Are liquid chromatography/electrospray tandem quadrupole fragmentation ratios unequivocal confirmation criteria? *Rapid Communications in Mass Spectrometry* **2009**, *23*, (7), 985-998.
4. George-Ares, A.; Clark, J., Aquatic toxicity of two Corexit® dispersants. *Chemosphere* **2000**, *40*, (8), 897-906.
5. Mitchell, F. M.; Holdway, D. A., The acute and chronic toxicity of the dispersants Corexit 9527 and 9500, water accommodated fraction (WAF) of crude oil, and dispersant enhanced WAF (DEWAF) to *Hydra viridissima* (green hydra). *Water Research* **2000**, *34*, (1), 343-348.
6. DeLeo, D. M.; Ruiz-Ramos, D. V.; Baums, I. B.; Cordes, E. E., Response of deep-water corals to oil and chemical dispersant exposure. *Deep Sea Research Part II: Topical Studies in Oceanography* **2016**, *129*, 137-147.
7. Cohen, J. H.; McCormick, L. R.; Burkhardt, S. M., Effects of dispersant and oil on survival and swimming activity in a marine copepod. *Bulletin of environmental contamination and toxicology* **2014**, *92*, (4), 381-387.
8. Rico-Martínez, R.; Snell, T. W.; Shearer, T. L., Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A® to the *Brachionus plicatilis* species complex (Rotifera). *Environmental Pollution* **2013**, *173*, 5-10.
9. Singer, M. M.; George, S.; Jacobson, S.; Lee, I.; Weetman, L. L.; Tjeerdema, R. S.; Sowby, M. L., Comparison of acute aquatic effects of the oil dispersant Corexit 9500 with those of other Corexit series dispersants. *Ecotoxicology and Environmental Safety* **1996**, *35*, (2), 183-189.

10. Kujawinski, E. B.; Soule, M. C. K.; Valentine, D. L.; Boysen, A. K.; Longnecker, K.; Redmond, M. C., Fate of Dispersants Associated with the Deepwater Horizon Oil Spill. *Environ Sci Technol* **2011**, *45*, (4), 1298-1306.
11. Gray, J. L.; Kanagy, L. K.; Furlong, E. T.; Kanagy, C. J.; McCoy, J. W.; Mason, A.; Lauenstein, G., Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. **2014**, *95*, 124-130.
12. Parker, A. M.; Ferrer, I.; Thurman, E. M.; Rosario-Ortiz, F. L.; Linden, K. G., Determination of COREXIT components used in the Deepwater Horizon cleanup by liquid chromatography-ion trap mass spectrometry. *Anal Methods-Uk* **2014**, *6*, (15), 5498-5502.
13. Hayworth, J. S.; Clement, T. P., Provenance of Corexit-related chemical constituents found in nearshore and inland Gulf Coast waters. *Marine pollution bulletin* **2012**, *64*, (10), 2005-2014.
14. Mathew, J.; Schroeder, D. L.; Zintek, L. B.; Schupp, C. R.; Kosempa, M. G.; Zachary, A. M.; Schupp, G. C.; Wesolowski, D. J., Dioctyl sulfosuccinate analysis in near-shore Gulf of Mexico water by direct-injection liquid chromatography-tandem mass spectrometry. *J Chromatogr A* **2012**, *1231*, 46-51.
15. Batchu, S. R.; Ramirez, C. E.; Gardinali, P. R., Stability of dioctyl sulfosuccinate (DOSS) towards hydrolysis and photodegradation under simulated solar conditions. *Science of the Total Environment* **2014**, *481*, 260-265.
16. Campo, P.; Venosa, A. D.; Suidan, M. T., Biodegradability of Corexit 9500 and dispersed South Louisiana crude oil at 5 and 25 C. *Environ Sci Technol* **2013**, *47*, (4), 1960-1967.
17. Glover, C. M.; Mezyk, S. P.; Linden, K. G.; Rosario-Ortiz, F. L., Photochemical degradation of Corexit components in ocean water. *Chemosphere* **2014**, *111*, 596-602.
18. Kover, S. C.; Rosario-Ortiz, F. L.; Linden, K. G., Photochemical fate of solvent constituents of Corexit oil dispersants. *Water research* **2014**, *52*, 101-111.
19. Bælum, J.; Borglin, S.; Chakraborty, R.; Fortney, J. L.; Lamendella, R.; Mason, O. U.; Auer, M.; Zemla, M.; Bill, M.; Conrad, M. E., Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. *Environmental microbiology* **2012**, *14*, (9), 2405-2416.
20. Brunswick, P.; Shang, D.; van Aggelen, G.; Buday, C.; Kim, M., Rapid and Sensitive LC/MS/MS Direct Injection Method for the Determination of Trace Level Corexit EC9500A Oil Dispersant in Seawater. *Environmental Forensics* **2015**, *16*, (4), 333-343.
21. Seidel, M.; Kleindienst, S.; Dittmar, T.; Joye, S. B.; Medeiros, P. M., Biodegradation of crude oil and dispersants in deep seawater from the Gulf of Mexico: Insights from ultra-high resolution mass spectrometry. *Deep Sea Research Part II: Topical Studies in Oceanography* **2016**, *129*, 108-118.
22. Deepwater Horizon oil spill: Final Programmatic Damage Assessment and Restoration Plan and Final Programmatic Environmental Impact Statement. Retrieved from <http://www.gulfspillrestoration.noaa.gov/restoration-planning/gulf-plan> Deepwater Horizon Natural Resource Damage Assessment Trustees (2016).
23. National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling. The Use of Surface and Subsea Dispersants During the BP Deepwater Horizon Oil Spill. **2011**.

24. Reddy, C. M.; Arey, J. S.; Seewald, J. S.; Sylva, S. P.; Lemkau, K. L.; Nelson, R. K.; Carmichael, C. A.; McIntyre, C. P.; Fenwick, J.; Ventura, G. T., Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. *Proceedings of the National Academy of Sciences* **2012**, *109*, (50), 20229-20234.
25. Paine, R. T.; Ruesink, J. L.; Sun, A.; Soulanille, E. L.; Wonham, M. J.; Harley, C. D.; Brumbaugh, D. R.; Secord, D. L., Trouble on oiled waters: lessons from the Exxon Valdez oil spill. *Annual Review of Ecology and Systematics* **1996**, *27*, (1), 197-235.
26. Jewett, S. C.; Dean, T. A.; Smith, R. O.; Blanchard, A., 'Exxon Valdez' oil spill: impacts and recovery in the soft-bottom benthic community in and adjacent to eelgrass beds. *Marine Ecology Progress Series* **1999**, *185*, 59-83.
27. National Research Council. Dispersant Oil Spill Efficacy and Effects. **2005**.
28. Reichert, M. D.; Walker, L. M., Interfacial tension dynamics, interfacial mechanics, and response to rapid dilution of bulk surfactant of a model oil–water-dispersant system. *Langmuir* **2013**, *29*, (6), 1857-1867.
29. Zhang, Z.; Avij, P.; Perkins, M. J.; Liyana-Arachchi, T. P.; Field, J. A.; Valsaraj, K. T.; Hung, F. R., Combined Experimental and Molecular Simulation Investigation of the Individual Effects of Corexit Surfactants on the Aerosolization of Oil Spill Matter. *The Journal of Physical Chemistry A* **2016**, *120*, (30), 6048-6058.
30. Riehm, D. A.; McCormick, A. V., The role of dispersants' dynamic interfacial tension in effective crude oil spill dispersion. *Marine pollution bulletin* **2014**, *84*, (1), 155-163.
31. Lessard, R. R.; DeMarco, G., The significance of oil spill dispersants. *Spill Science & Technology Bulletin* **2000**, *6*, (1), 59-68.
32. Place, B.; Anderson, B.; Mekebri, A.; Furlong, E. T.; Gray, J. L.; Tjeerdema, R.; Field, J., A role for analytical chemistry in advancing our understanding of the occurrence, fate, and effects of Corexit oil dispersants. In ACS Publications: 2010.
33. Ramirez, C. E.; Batchu, S. R.; Gardinali, P. R., High sensitivity liquid chromatography tandem mass spectrometric methods for the analysis of dioctyl sulfosuccinate in different stages of an oil spill response monitoring effort. *Anal Bioanal Chem* **2013**, *405*, (12), 4167-4175.
34. Borisov, O. V.; Ji, J. A.; Wang, Y. J.; Vega, F.; Ling, V. T., Toward understanding molecular heterogeneity of polysorbates by application of liquid chromatography–mass spectrometry with computer-aided data analysis. *Analytical chemistry* **2011**, *83*, (10), 3934-3942.
35. Erdem, N. S.; Alawani, N.; Wesdemiotis, C., Characterization of polysorbate 85, a nonionic surfactant, by liquid chromatography vs. ion mobility separation coupled with tandem mass spectrometry. *Analytica chimica acta* **2014**, *808*, 83-93.
36. Operational Science Advisory Team Unified Area Command. Summary Report for Sub-Sea and Sub-Surface Oil and Dispersant Detection: Sampling and Monitoring. **2010**.
37. Forth, H. P.; Mitchelmore, C. L.; Morris, J. M.; Lipton, J., Characterization of oil and water accommodated fractions used to conduct aquatic toxicity testing in support of the Deepwater Horizon oil spill natural resource damage assessment. *Environmental Toxicology and Chemistry* **2015**.

38. Singer, M.; Aurand, D.; Bragin, G.; Clark, J.; Coelho, G.; Sowby, M.; Tjeerdema, R., Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. *Marine Pollution Bulletin* **2000**, *40*, (11), 1007-1016.
39. Özhan, K.; Miles, S. M.; Gao, H.; Bargu, S., Relative Phytoplankton growth responses to physically and chemically dispersed South Louisiana sweet crude oil. *Environmental monitoring and assessment* **2014**, *186*, (6), 3941-3956.
40. Anderson, J. A.; Kuhl, A. J.; Anderson, A. N., Toxicity of oil and dispersed oil on juvenile mud crabs, *Rhithropanopeus harrisi*. *Bulletin of environmental contamination and toxicology* **2014**, *92*, (4), 375-380.
41. Bejarano, A. C.; Clark, J. R.; Coelho, G. M., Issues and challenges with oil toxicity data and implications for their use in decision making: A quantitative review. *Environmental toxicology and chemistry* **2014**, *33*, (4), 732-742.
42. Adams, J.; Swezey, M.; Hodson, P. V., Oil and oil dispersant do not cause synergistic toxicity to fish embryos. *Environmental toxicology and chemistry* **2014**, *33*, (1), 107-114.
43. Camilli, R.; Reddy, C. M.; Yoerger, D. R.; Van Mooy, B. A.; Jakuba, M. V.; Kinsey, J. C.; McIntyre, C. P.; Sylva, S. P.; Maloney, J. V., Tracking hydrocarbon plume transport and biodegradation at Deepwater Horizon. *Science* **2010**, *330*, (6001), 201-204.
44. Barsamian, A. L.; Perkins, M. J.; Field, J. A.; Blakemore, P. R., Regioselective syntheses of [13C] 4-labelled sodium 1-carboxy-2-(2-ethylhexyloxycarbonyl) ethanesulfonate and sodium 2-carboxy-1-(2-ethylhexyloxycarbonyl) ethanesulfonate from [13C] 4-maleic anhydride. *Journal of Labelled Compounds and Radiopharmaceuticals* **2014**, *57*, (5), 397-401.
45. Garcia, M.; Campos, E.; Marsal, A.; Ribosa, I., Biodegradability and toxicity of sulphonate-based surfactants in aerobic and anaerobic aquatic environments. *Water Research* **2009**, *43*, (2), 295-302.
46. Hales, S. G., Biodegradation of the anionic surfactant dialkyl sulphosuccinate. *Environmental toxicology and chemistry* **1993**, *12*, (10), 1821-1828.
47. Passow, U., Formation of rapidly-sinking, oil-associated marine snow. *Deep Sea Research Part II: Topical Studies in Oceanography* **2014**.
48. Vonk, S. M.; Hollander, D. J.; Murk, A. J., Was the extreme and wide-spread marine oil-snow sedimentation and flocculent accumulation (MOSSFA) event during the Deepwater Horizon blow-out unique? *Marine Pollution Bulletin* **2015**, *100*, (1), 5-12.
49. White, H. K.; Lyons, S. L.; Harrison, S. J.; Findley, D. M.; Liu, Y.; Kujawinski, E. B., Long-Term Persistence of Dispersants following the Deepwater Horizon Oil Spill. *Environ Sci Technol Lett* **2014**, *1*, (7), 295-299.
50. Monahan, E. C.; Mac Niocaill, G., *Oceanic Whitecaps: And Their Role in Air-Sea Exchange Processes*. Springer Science & Business Media: 2012; Vol. 2.
51. Ehrenhauser, F. S.; Avij, P.; Shu, X.; Dugas, V.; Woodson, I.; Liyana-Arachchi, T.; Zhang, Z.; Hung, F. R.; Valsaraj, K. T., Bubble bursting as an aerosol generation mechanism during an oil spill in the deep-sea environment: laboratory experimental demonstration of the transport pathway. *Environmental Science: Processes & Impacts* **2014**, *16*, (1), 65-73.

CHAPTER 2 - COREXIT SURFACTANT BIODEGRADATION, HYDROLYSIS AND AQUEOUS PHASE COMPOSITION AFTER CEWAF FORMATION

Matt J. Perkins¹, Sara Kleindienst², Sairah Malkin², Samantha B. Joye², Jennifer A. Field¹

¹ Department of Environmental and Molecular Toxicology, Oregon State University,
Corvallis, OR 97331

² Department of Marine Sciences, University of Georgia, Athens, GA 30605

In preparation for submission

2.1 Abstract

In response to the Deepwater Horizon oil spill of 2010, approximately 7.1 million L of Corexit 9527A and 9500A (Corexit) were applied to surface-oil slicks and directly at the wellhead. A single component, DOSS, was documented to persist in a deep-water plume. Studies on the degradation of the Corexit surfactants using microbial cultures relevant to the Gulf are limited and are primarily focused on DOSS. Furthermore, DOSS has been suggested to undergo transformation to EHSS under biotic and abiotic conditions. High purity standards of EHSS were synthesized and the time course biodegradation of all of the individual surfactant components of Corexit and EHSS were quantified in Gulf deep-water microcosms by LC-MS/MS. Surfactant biodegradation was evaluated using Corexit-only and CEWAF ($\pm$ nutrients) treatments. The abiotic hydrolysis of DOSS, or lack thereof, was also quantified at 5° and 25° C in Corexit-only microcosms. The abiotic hydrolysis of DOSS was not observed in this study. Rapid degradation of the nonionic surfactants was observed in the Corexit-only treatment but they were not detected in time zero CEWAF samples. No significant change in DOSS or EHSS were observed in the Corexit-only treatment, but microbial degradation of DOSS did occur (33-41%) in the CEWAF ($\pm$ nutrients) treatments. No significant difference in EHSS was observed, indicating that other transformation intermediates are formed during DOSS biodegradation. In addition, this finding indicates that EHSS is not biodegraded under the test conditions. In contrast to previous reports, no significant abiotic loss of DOSS was observed. However, significant differences in the surfactant composition between treatments at time zero demonstrate the criticality of quantifying the individual surfactant components in time zero samples. Furthermore, these results demonstrate that

Corexit-only solutions are not suitable controls for determining the contribution of the Corexit surfactants to the toxicity of CEWAF.

2.2 Introduction

The Deepwater Horizon (DWH) oil spill resulted in the largest accidental marine oil spill in international history,¹ was the first deep-sea blowout to occur (~1500 m),² an unprecedented volume (7 million L) of chemical-oil dispersant (dispersant) was applied,^{3,4} and the first deep-sea application of dispersant (2.9 million L) occurred.⁵ Dispersant is applied to spilled oil primarily to increase the dispersion of surface oil into the water column, thereby reducing the potential impact of onshore oiling.⁶ Increased dispersion of oil occurs because the surfactant components reduce the surface tension of the oil, allowing smaller oil droplets to form under breaking waves.⁷ Smaller oil droplets are more easily retained and dispersed vertically in the water column due to their lower buoyancy. These droplets also form under lower energy conditions.³ Additionally, smaller droplets of oil have a larger surface area to volume ratio.⁸ Therefore, a volume of oil has a larger net surface area when chemically dispersed, potentially leading to higher rates of microbial degradation.^{4,9} However, the effect of dispersant on the rate of crude oil biodegradation is controversial and still unfolding.^{3,10}

Two dispersant formulations were applied in the Gulf of Mexico (Gulf) in response to DWH: Corexit 9527A and Corexit 9500A.³ The surfactant components include the anionic surfactant Bis-(2-ethylhexyl)sulfosuccinate (DOSS, 10-22%) and the nonionic surfactants Span 80 (2.7-4.4%), Tween 80 (11-18%) and Tween 85 (4.3-4.6%) (Figure A1.1 and A1.2). The surfactant components are similar in their mass percent

contribution between the two formulations.¹¹⁻¹³ Impurities also exist in the off-the-shelf formulations of Corexit, including α/β -ethylhexylsulfosuccinate (EHSS) at 0.28% in Corexit 9500A.¹² EHSS was identified as a microbial and abiotic hydrolysis product of DOSS.^{14, 15}

In the aftermath of DWH, environmental monitoring of Corexit largely focused on a single component, DOSS.^{12, 13, 16-18} In particular, DOSS was characterized^{12, 16} in a prominent deep-water (~1100 m) hydrocarbon plume¹⁹ that extended more than 250 km southwest of the DWH.²⁰ DOSS observed in the plume was determined to have originated from the application of Corexit at the wellhead. Furthermore, DOSS did not undergo appreciable degradation before being diluted below detection over 6 weeks.¹⁶ The persistence of DOSS contradicts assumptions regarding the amenability of the individual components of Corexit to degradation under conditions relevant to the Gulf.^{4, 5}

In contrast to field observations, Campo et al., 2013 used microbial cultures isolated from Gulf waters to conduct a laboratory microcosm study using Corexit-only and chemically dispersed Corexit-crude-oil mixtures (chemically enhanced water accommodated fraction, CEWAF).²¹ Common methods for the creation of a CEWAF involve slowly stirring a mixture of Corexit (or other oil dispersant) oil and seawater for a period of days. This mixture is allowed to rest for an extended period and then the aqueous fraction, or chemically enhanced water accommodated fraction, is separated and the free-phase oil is discarded. Standard methods exist for creating CEWAF are necessary to generate solutions in which the hydrocarbon constituents are reproducible and comparable.^{22,23} Campo et al., reported significant degradation of DOSS (61-99%)

over 28 days at 5 and 25° C. These authors also observed the presence of EHSS but did not quantify EHSS or any components of Corexit other than DOSS.

To date laboratory studies on the degradation of Corexit, the impact of Corexit on crude oil biodegradation and the impact of Corexit on the Gulf microbial communities are still limited. Therefore, microcosm experiments were conducted using Gulf deep-water to generate Corexit 9500-only (i.e. Corexit stirred in seawater in the absence of oil), physically dispersed oil, or a water accommodated fraction (WAF), and CEWAF solutions. Standard methods for creating a WAF are the same as for a CEWAF, except that Corexit (or other oil dispersant) is not added to the oil-seawater mixture. The time course degradation of all of the surfactant components of Corexit was quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS). Additionally, hydrocarbon biodegradation was quantified by GC-MS and the rate of degradation of representative hydrocarbons was determined using a radio tracer assay. The bulk and taxonomic response of the microbial community was also monitored. The impact of Corexit 9500 on hydrocarbon degradation and the microbial community, as well as the surfactant data necessary to support those results, are presented in the study of Kleindienst et al.¹⁰

In the present study, the surfactant data is examined in greater detail. Specifically, a high purity standard of α -EHSS and a $^{13}\text{C}_4$ -labelled analog of DOSS were synthesized at OSU,²⁴ used to quantify EHSS and to evaluate the mass balance, or lack of mass balance, of DOSS. The primary biodegradation of the nonionic surfactants is also quantified. Experiments were conducted to clarify observations made by Campo et al.; namely, that DOSS undergoes rapid abiotic hydrolysis at elevated temperatures (i.e. 25°

C). The results of the present study indicates that DOSS does not readily undergo abiotic hydrolysis. Rather, the abiotic loss of DOSS observed by Campo et al., may result from the addition of the biocide, sodium azide. Additionally, in the present study, significant differences in the aqueous phase surfactant composition were observed between the Corexit-only and CEWAF solutions at time zero. These compositional differences are not currently addressed in the CEWAF literature^{22, 23, 25-28} and these findings imply that surfactant exposure concentrations depend on how Corexit is prepared.

2.3 Experimental

2.3.1 Chemicals and Standards. Solid (98.1%) bis-(2-ethylhexyl) sodium sulfosuccinate (DOSS); liquid standards of sorbitan monooleate (Span 80; purity: 70.5%), sorbitan monooleate polyethoxylate (Tween 80; purity:74%), and sorbitan monooleate polyethoxylate (Tween 85; purity:67%); as well as high purity ammonium acetate were obtained from Sigma Aldrich (Saint Louis, MO, USA). MS-grade isopropanol and acetonitrile were obtained from Fisher Scientific (Pittsburg, PA, USA). A standard of ¹³C₄-DOSS was provided by Ed Furlong and James Gray of the United States Geological Survey National Water Quality Laboratory (Denver, CO, USA) that had been synthesized by Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). High purity standards of α -ethylhexylsulfosuccinate (α -EHSS) and the ¹³C₄- α -EHSS labelled analog were synthesized in house.²⁴ Corexit 9500A was obtained from Nalco Environmental Solutions (Sugar Land, TX, USA). Laboratory 18-M Ω , deionized (DI) water was obtained by an in-house Millipore Synergy unit with an LC-Pak polisher (EMD Millipore Corp, Billerica, MA). Instant Ocean was purchased at PetSmart (Phoenix, AZ, USA). Parent stock

standards were prepared in methanol (DOSS), isopropanol (nonionics) or water (EHSS) within 30 days of analysis. Analytical standards were prepared in a synthetic seawater and isopropanol solution (75:25, SW:IPA).

2.3.2 Seawater Collection. Seawater was collected in the Gulf at depth (1,178 m) near an active natural hydrocarbon seep as described previously.¹⁰

2.3.3 Corexit Only and CEWAF Solutions for Microcosms. The setup of all solutions and sampling occurred at the University of Georgia. Three experimental treatments were employed: Corexit-only, CEWAF, and CEWAF + nutrients. The experimental setup was described in detail in Kleindienst et al.¹⁰ Briefly, the Corexit-only solution was created by adding 0.015 L of Corexit 9500 to 0.85 L of sterile seawater. The solution was stirred at 600 rpm for 48 h in the dark at room temperature in 1 L glass bottles. The solution was allowed to rest for 1 h. Corexit was visible on the sides of these jars indicating incomplete solvation of Corexit (Figure A1.3A). Next, 0.00156 L of the aqueous phase of this initial solution (i.e. avoiding Corexit on the sides of the glass) was subsampled and diluted with sterile seawater to make a final volume of 0.4 L. This 0.4 liters was further diluted with an additional 1.4 L of live seawater to make a final nominal concentration of $\sim 15.3 \text{ mg L}^{-1}$ Corexit. The CEWAF ($\pm$ nutrients) treatments were created by adding 0.015 L of Corexit 9500 and 0.15 L of crude Macondo surrogate oil to 0.85 L of sterile seawater. The solution was stirred and allowed to rest as described above. Free-phase oil was visible at the surface of the aqueous phase (Figure A1.3B). 0.00326 L of the aqueous phase (i.e. the free-phase oil was discarded) of this initial solution was subsampled and diluted with sterile seawater to make a final volume of 0.4 L. This 0.4

liters was further diluted with an additional 1.4 L of live seawater to make a final nominal concentration of $\sim 32 \text{ mg L}^{-1}$ Corexit.

2.3.4 Microcosm Setup and Sampling. Microcosms were kept on a roller table in the dark in a cold room at 8° until they were destructively harvested at time zero, 7, 17, 28, and 42 d (Corexit-only and CEWAF) or time zero, 7 and 42 days (CEWAF + nutrients). Seawater was diluted with isopropanol (75:25, SW:IPA) and frozen at -20°C . Samples were shipped to Oregon State University on dry ice where they were stored at -20° until analysis.

2.3.5 Abiotic Controls. Two sets of abiotic controls were conducted at a later time and by different personnel. The Corexit only solution for the abiotic controls was created as described above. The abiotic controls were destructively harvested at time zero, 7 and 39 days and time zero, 7, and 24 days for the experiments conducted at 8° and 25°C , respectively. For the experiment at 8°C , the Corexit-only solution was created by diluting 0.5 mL of Corexit 9500A in seawater to reach a total volume of 2 L ($\sim 250 \text{ mg L}^{-1}$ Corexit nominal concentration). For the experiment at 25°C , the Corexit-only solution was created by diluting 12.5 μL of Corexit 9500A in seawater to reach a total volume of 2 L ($\sim 6.25 \text{ mg L}^{-1}$ Corexit nominal concentration).

2.3.6 Additional Corexit-only and CEWAF ($\pm$ nutrients) solutions.

The second Corexit-only solution was created by adding 0.5 mL of Corexit 9500 to 2 L of live seawater. The solution was stirred at 600 rpm for 48 h in the dark at room temperature. The solution was allowed to rest for 4 h and then the aqueous phase was siphoned off the bottom. to make a final nominal concentration of $\sim 250 \text{ mg L}^{-1}$ Corexit.

The CEWAF ($\pm$ nutrients) treatments were created by adding 5 mL of Corexit 9500 and 100 mL of crude Macondo surrogate oil to 2000 mL of sterile seawater. The solution was stirred and allowed to rest as described above. Then 100 mL was subsampled and added to 1900 mL of live seawater to yield a final nominal concentration of $\sim 125 \text{ mg L}^{-1}$.

2.3.7 Liquid Chromatography. Chromatographic separations were performed on an Agilent 1100 (Agilent Technologies, Inc., Santa Clara, CA) as described in Place et al.,¹² with minor modification. Briefly, an Agilent Proshell 120 EC-C18 guard column (4.6 mm ID_5 mm length_2.7 μm particle size) was placed in front of an Agilent XDB-C18 (DOSS) or an Agilent XDB-C8 (Tween 80 and Tween 85) analytical column (4.6 mm ID_20 mm length_3.5 μm particle size; C8 and C18). Also, during the analysis of the nonionic surfactants, the mobile phase was directed through an Agilent thermostatted column compartment (G1316A) and heated to 40 $^{\circ}\text{C}$.

Additionally, the initial flow rate was increased to 1 mL min^{-1} during sample loading and washing of the non-volatile salts from the column (first 5.6 minutes) without degrading the peak shape or percent recovery of the analytes of interest. The gradient was further modified such that the 97.5 % acetonitrile was held for 3 min before returning to 5 % acetonitrile for 6 min. The flow rate was 1 mL min^{-1} for the first 5.6 min, 0.5 mL min^{-1} for 5 min, and 1.0 mL min^{-1} for 6 minutes. The timing of the main-pass-by-pass valve switching and divert valve switching, as described by Place et al.,¹² was adjusted to reflect changes in the flow rate and gradient. These chromatographic changes were made to achieve better peak symmetry and to significantly shorten the run time of the analysis.

2.3.8 Tandem Mass Spectrometry. Mass spectrometric detection was performed on a Waters Micromass Quattro Mass Spectrometer (Waters Corporation, Milford, MA). Tandem quadrupole mass spectrometric analysis was performed as described in Place et al.,¹² except that the analysis of Span 80 was not acquired concurrently with that of Tween 80 and Tween 85. A separate analysis was performed for Span 80 (individual) and Tween 80 and Tween 85 (concurrent) to allow for a greater number of scans for Span 80, which would otherwise be limited by the long scan times of the precursor ion scan used to quantify the Tweens. Also, the mass range of the precursor ion scan used to quantify Tween 80 and Tween 85 was truncated to focus on the oligomers of greatest abundance in these complex mixtures (m/z 640-930). This truncation was implemented to decrease the individual scan time of each precursor ion scan, increasing the total scan time for the high abundance oligomers, increasing sensitivity.

Calibration curves consisted of at least 5 standards and required a correlation coefficient of 0.99 or greater to be used for quantification. All calibration curves were 1/X weighted, and standards whose calculated concentrations were beyond 20% of the intended concentration were removed from the calibration curve calculation. Calibration curves spanned from the lower limit of quantification (LLOQ) to the upper limit of quantification (ULOQ): for DOSS (0.2-25 $\mu\text{g L}^{-1}$), α -/ β -EHSS (0.2-23 $\mu\text{g L}^{-1}$), Span 80 (60-300 $\mu\text{g L}^{-1}$), Tween 80 (60-300 $\mu\text{g L}^{-1}$), and Tween 85 (60-300 $\mu\text{g L}^{-1}$). Each calibration standard was spiked to give a final concentration of 500 ng L^{-1} $^{13}\text{C}_4$ -DOSS and 500 ng L^{-1} $^{13}\text{C}_4$ - α -EHSS. Blank and check standards (34) were used for quality control purposes. Standards for DOSS and EHSS fell within 20% of the spiked

concentration and the nonionic Corexit surfactants fell within 35% of the spiked concentration. All blank QC fell below the limit of detection.

2.4 Results and Discussion

2.4.1 Degradation of the Corexit surfactants. In the dispersant-only treatment, no significant difference ($P < 0.5$) in the concentration of DOSS or EHSS was observed over 42 days (Figure 2.1, Table A1.1). EHSS has been reported as a transformation product of the microbial and abiotic hydrolysis of DOSS.^{14, 15} The nonionic surfactants were consumed within 1 week driving concentrations below the limit of detection (LOD, $20 \mu\text{g L}^{-1}$) (Figure 2.1, Table A1.1). In the CEWAF ($\pm$ nutrients) treatments, DOSS decreased significantly (33-41%, $P < 0.05$) over 42 days (Figure 2.1 Table A1.1). No significant change in EHSS concentration was observed in CEWAF ($\pm$ nutrients) treatments indicating that other transformation intermediates are formed during DOSS biodegradation (Figure 2.1, A1.1). The nonionic surfactants were not observed above the LOD at time zero in CEWAF ($\pm$ nutrients) treatments (Figure 2.1, Table A1.1) indicating that the nonionic surfactants partitioned into the free-phase oil and were discarded when the aqueous phase was separated. Therefore, the nonionic surfactants were likely not present in CEWAF during the incubation.

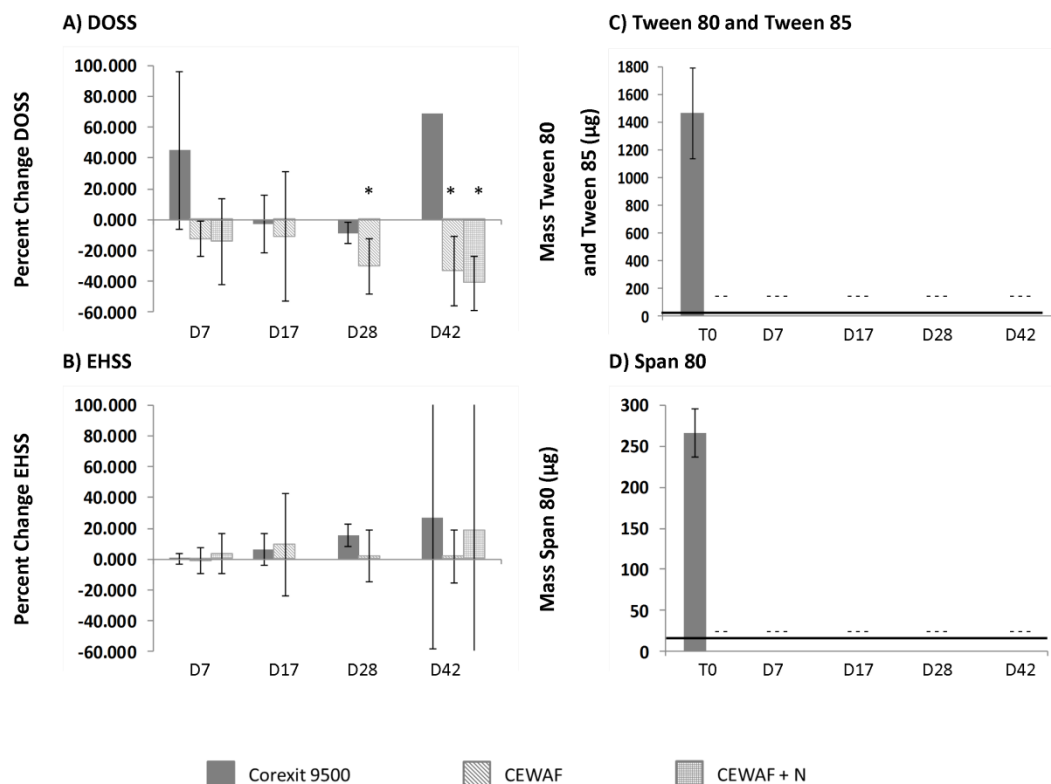


Figure 2.1. DOSS, EHSS, Span 80, and Tween 80 and Tween 85 in the dispersant-only and CEWAF ($\pm$ nutrients) treatments within 42 days of incubation. Mean percent change in DOSS (a) and EHSS (b) relative to time zero samples as well as mean mass of total Tweens (Tween 80 and Tween 85; c) and Span 80 (d). Error bars represent 95% confidence intervals ($n = 3$). Asterisk (*) indicates a statistically significant change (Student's t-test, $\alpha = 0.05$) and negative (-) indicates an observation was below the limit of detection for the nonionic surfactants (36 μg , 20 $\mu\text{g/L}$), illustrated as black line.

An abiotic killed control was conducted at 8° C using Corexit 9500 only. No significant difference (< 0.05) in the aqueous phase concentration was observed over 39

days for DOSS, EHSS and Tween 80 and Tween 85, indicating that the consumption of the surfactant components of Corexit was due to microbial action (Table A1.2). An analysis of Span 80 was not conducted on the abiotic control samples. Campo et al., reported much higher consumption of DOSS over 42 days for both Corexit 9500-only (61%) and CEWAF treatments (>98%) under similar experimental conditions.²¹ Campo et al also observed limited abiotic losses of DOSS at a similar temperature (i.e. 5° C). Campo et al., did not include the nonionic surfactants in their analysis, thus preventing comparison.

2.4.2 Implications of Aqueous Phase Surfactant Composition.

The surfactant components of Corexit 9500 DOSS, Span 80 and Tween 80 and Tween 85 represent 18, 4.4, and 22.6% of Corexit by mass, respectively.¹² EHSS was previously found to contribute 0.28% to the mass of Corexit 9500.¹² The surfactant components in the off-the-shelf formulation of Corexit were ratioed to that of EHSS (e.g. the ratio of DOSS to EHSS is $18/0.28 = 64$) (Table A1.3). The individual surfactant components quantified at time zero in Corexit-only and CEWAF treatments were ratioed to that of EHSS (Table A1.4). The ratio of the off-the-shelf Corexit formulation was compared to the ratio in the Corexit-only and CEWAF treatments to determine if the aqueous phase composition of the experimental treatments was consistent with whole Corexit. Additionally, the concentration of EHSS was compared to the concentration calculated using a bulk dilution factor (i.e. $C_1V_1 = C_2V_2$) to quantify the fraction of EHSS incorporated into aqueous solution (Table A1.5).

The ratio of DOSS, Span 80 and Tween 80 and Tween 85 to EHSS at time zero in the Corexit-only treatment was in good agreement with the ratio in the off-the-shelf-Corexit formulation (Figure 3A, Table A1.4). Confirming this observation, similar ratios were observed for DOSS and Tween 80 and Tween 85 in the abiotic controls (Table A1.4). These results indicate that the composition of Corexit was maintained when Corexit was added to seawater, in the absence of oil. However, the initial concentration of EHSS in the Corexit-only treatment and abiotic controls accounted for only 2-44% of the Corexit added to seawater, indicating that much less Corexit was incorporated into solution than predicted from the bulk dilution calculations (Table A1.5). This missing mass can be explained by the presence of Corexit on the sides of the glassware used to mix the Corexit and seawater (Figure A1.3A). A conceptual model is presented in Figure A1.4.

In contrast to the Corexit-only treatment, the ratio of the individual surfactants to EHSS in the CEWAF ($\pm$ nutrients) treatments was significantly different from that of whole Corexit. The surfactant composition of the aqueous phase was enriched in the most hydrophilic constituent, EHSS, depleted in DOSS and the nonionic surfactants were not observed above the LOD (Figure 3A, Table A1.1). This suggests that a fraction of DOSS (>98%) and all of the Span 80 and Tween 80 and Tween 85 (>99%) associated with the free-oil phase during CEWAF formation and that this mass of surfactants was discarded along with the free-phase oil when the aqueous phase was separated (see conceptual model, Figure A1.4). The concentration of EHSS accounted for only 25% of the Corexit added to CEWAF (Table A1.6), indicating that a fraction of the available EHSS (75%) in the CEWAF treatments associated with the free oil phase.

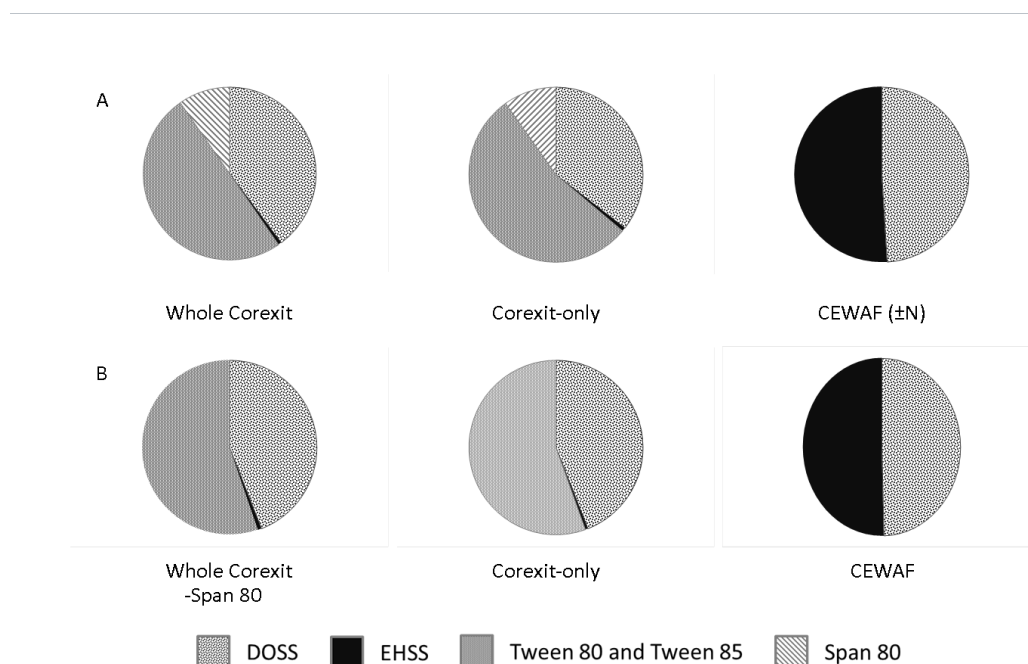


Figure 2.2. (A) The surfactant composition of whole Corexit 9500 and the time zero mean surfactant composition of the Corexit-only and CEWAF ($\pm$ nutrients) treatments. **(B)** The composition of DOSS, EHSS and Tween 80 and Tween in whole Corexit 85 (Span 80 was omitted) and additional Corexit-only and CEWAF initial solutions.

To confirm these observations an additional Corexit only and CEWAF ($\pm$ nutrients) solution was created (A1.6). An analysis of Span 80 was not conducted on these samples. In the second Corexit-only solution, the ratio of DOSS and Tween 80 and Tween 85 to EHSS agreed with the ratio in whole Corexit (Figure 3B, Table A1.4) and the concentration of EHSS in the Corexit-only solution accounted for only 5 percent of the Corexit added to seawater (Table A.1.5). These results confirm that the composition of Corexit was maintained when added to seawater but that much less of the individual surfactant components were incorporated into solution than predicted from bulk dilution calculations.

In the second CEWAF solution, the surfactant composition was enriched in EHSS, depleted in DOSS and Tween 80 and Tween 85 were not observed above the LOD (Figure 3B, Table A1.6). This observation confirms that a fraction of available DOSS (98%), and possibly all of the available Tween 80 and Tween 85 (>99%) associated with the free-phase oil during CEWAF formation (Table A1.6). Similar to the Corexit only solution, the concentration of EHSS accounted for only 3.6% of the Corexit added to CEWAF indicating that a fraction of the available EHSS (93.1%) in the CEWAF treatments associated with the free oil phase (Table A1.5).

2.4.3. Abiotic hydrolysis of DOSS at 25° C. An additional abiotic control was conducted over 24 days at 25° C and the concentration of DOSS and EHSS were determined. No significant difference in the concentration of DOSS or EHSS (Table A1.7) was observed over the course of the incubation, indicating that the abiotic hydrolysis of DOSS did not occur in this experiment. This observation lies in stark contrast to that of Campo et al., who reported abiotic losses of DOSS greater than 70% within 28 days at 25° C and attributed these losses to the abiotic hydrolysis of DOSS.²¹ This difference in the apparent stability of DOSS may be the result of differences in the method of sterilization employed. In the present study, seawater and Corexit were sterilized by double pasteurization. No significant difference was observed in the concentration of DOSS, EHSS, or Tween 80 and Tween 85 before and after pasteurization (Table A1.2). However, Campo et al., employed sodium azide at a concentration of 0.5 mg L⁻¹ as a biocide. Sodium azide is a strong nucleophile and may have promoted the degradation of DOSS by nucleophilic substitution.

2.5 Implications

DOSS underwent limited biodegradation under the experimental conditions presented here. Negligible degradation of DOSS was also observed in deep-waters of the Gulf during the DWH emergency response.¹⁶ Therefore, the experimental conditions presented here may be more representative of the field conditions that occurred in response to spilled DWH oil than those presented by other studies. The nonionic surfactants underwent rapid primary biodegradation under the aerobic conditions implemented here. Only limited monitoring of the nonionic surfactants in the water column occurred during DWH, preventing comparison of the results of the present study to field observations.

The present study documents important considerations for experimental design. Here, lower surfactant concentration in CEWAF relative to the Corexit-only treatment occurred because the surfactant components partitioned out of seawater and associated with the free oil phase and were discarded when the aqueous phase (i.e. CEWAF) was separated from the seawater phase. The results of the present study underscore the fact that Corexit is a complex mixture, the individual components of which have unique solubility and polarity. As a result the individual components exhibit different partitioning behavior, requiring the quantification the individual components in time zero solutions rather than relying on calculated concentrations based on dilution factors. Significant differences in the aqueous phase surfactant composition imply 1) Corexit-only solutions are not appropriate controls for studies attempting to disentangle the toxicity of the surfactant components of CEWAF from that of the hydrocarbon components because they contain much higher surfactant concentrations than do

CEWAFs, which are used in microcosm experiments and 2) the transport of Corexit in marine systems should not be treated as a bulk solution. Additionally, sterilization of seawater for abiotic controls was achieved by pasteurization. This and similar techniques may be preferable to the addition of chemical biocides, such as sodium azide...

2.6 Literature Cited

1. National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling. The Use of Surface and Subsea Dispersants During the BP Deepwater Horizon Oil Spill. **2011**.
2. Reddy, C. M.; Arey, J. S.; Seewald, J. S.; Sylva, S. P.; Lemkau, K. L.; Nelson, R. K.; Carmichael, C. A.; McIntyre, C. P.; Fenwick, J.; Ventura, G. T., Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. *Proceedings of the National Academy of Sciences* **2012**, *109*, (50), 20229-20234.
3. National Research Council. Dispersant Oil Spill Efficacy and Effects. **2005**.
4. Operational Science Advisory Team Unified Area Command. Summary Report for Sub-Sea and Sub-Surface Oil and Dispersant Detection: Sampling and Monitoring. **2010**.
5. Deepwater Horizon oil spill: Final Programmatic Damage Assessment and Restoration Plan and Final Programmatic Environmental Impact Statement. Retrieved from <http://www.gulfspillrestoration.noaa.gov/restoration-planning/gulf-plan> **Deepwater Horizon Natural Resource Damage Assessment Trustees (2016)**.
6. Lessard, R. R.; DeMarco, G., The significance of oil spill dispersants. *Spill Science & Technology Bulletin* **2000**, *6*, (1), 59-68.
7. Reichert, M. D.; Walker, L. M., Interfacial tension dynamics, interfacial mechanics, and response to rapid dilution of bulk surfactant of a model oil–water–dispersant system. *Langmuir* **2013**, *29*, (6), 1857-1867.
8. Prince, R. C., Oil Spill Dispersants: Boon or Bane? *Environ Sci Technol* **2015**, *49*, (11), 6376-6384.
9. Prince, R. C.; Butler, J. D., A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil. *Environmental Science and Pollution Research* **2014**, *21*, (16), 9506-9510.
10. Kleindienst, S.; Seidel, M.; Ziervogel, K.; Grim, S.; Loftis, K.; Harrison, S.; Malkin, S. Y.; Perkins, M. J.; Field, J.; Sogin, M. L., Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. *Proceedings of the National Academy of Sciences* **2015**, 201507380.
11. Ramirez, C. E.; Batchu, S. R.; Gardinali, P. R., High sensitivity liquid chromatography tandem mass spectrometric methods for the analysis of dioctyl sulfosuccinate in different stages of an oil spill response monitoring effort. *Anal Bioanal Chem* **2013**, *405*, (12), 4167-4175.
12. Place, B. J.; Perkins, M. J.; Sinclair, E.; Barsamian, A. L.; Blakemore, P. R.; Field, J. A., Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. *Deep Sea Research Part II: Topical Studies in Oceanography* **2014**.

13. Gray, J. L.; Kanagy, L. K.; Furlong, E. T.; Kanagy, C. J.; McCoy, J. W.; Mason, A.; Lauenstein, G., Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. **2014**, *95*, 124-130.
14. Garcia, M.; Campos, E.; Marsal, A.; Ribosa, I., Biodegradability and toxicity of sulphonate-based surfactants in aerobic and anaerobic aquatic environments. *Water Research* **2009**, *43*, (2), 295-302.
15. Hales, S. G., Biodegradation of the anionic surfactant dialkyl sulphosuccinate. *Environmental toxicology and chemistry* **1993**, *12*, (10), 1821-1828.
16. Kujawinski, E. B.; Soule, M. C. K.; Valentine, D. L.; Boysen, A. K.; Longnecker, K.; Redmond, M. C., Fate of Dispersants Associated with the Deepwater Horizon Oil Spill. *Environ Sci Technol* **2011**, *45*, (4), 1298-1306.
17. Hayworth, J. S.; Clement, T. P., Provenance of Corexit-related chemical constituents found in nearshore and inland Gulf Coast waters. *Marine pollution bulletin* **2012**, *64*, (10), 2005-2014.
18. Mathew, J.; Schroeder, D. L.; Zintek, L. B.; Schupp, C. R.; Kosempa, M. G.; Zachary, A. M.; Schupp, G. C.; Wesolowski, D. J., Dioctyl sulfosuccinate analysis in near-shore Gulf of Mexico water by direct-injection liquid chromatography-tandem mass spectrometry. *J Chromatogr A* **2012**, *1231*, 46-51.
19. Camilli, R.; Reddy, C. M.; Yoerger, D. R.; Van Mooy, B. A.; Jakuba, M. V.; Kinsey, J. C.; McIntyre, C. P.; Sylva, S. P.; Maloney, J. V., Tracking hydrocarbon plume transport and biodegradation at Deepwater Horizon. *Science* **2010**, *330*, (6001), 201-204.
20. Valentine, D. L.; Fisher, G. B.; Bagby, S. C.; Nelson, R. K.; Reddy, C. M.; Sylva, S. P.; Woo, M. A., Fallout plume of submerged oil from Deepwater Horizon. *Proceedings of the National Academy of Sciences* **2014**, *111*, (45), 15906-15911.
21. Campo, P.; Venosa, A. D.; Suidan, M. T., Biodegradability of Corexit 9500 and dispersed South Louisiana crude oil at 5 and 25 C. *Environ Sci Technol* **2013**, *47*, (4), 1960-1967.
22. Singer, M.; Aurand, D.; Bragin, G.; Clark, J.; Coelho, G.; Sowby, M.; Tjeerdema, R., Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. *Marine Pollution Bulletin* **2000**, *40*, (11), 1007-1016.
23. Forth, H. P.; Mitchelmore, C. L.; Morris, J. M.; Lipton, J., Characterization of oil and water accommodated fractions used to conduct aquatic toxicity testing in support of the Deepwater Horizon oil spill natural resource damage assessment. *Environmental Toxicology and Chemistry* **2015**.
24. Barsamian, A. L.; Perkins, M. J.; Field, J. A.; Blakemore, P. R., Regioselective syntheses of [13C] 4-labelled sodium 1-carboxy-2-(2-ethylhexyloxycarbonyl) ethanesulfonate and sodium 2-carboxy-1-(2-ethylhexyloxycarbonyl) ethanesulfonate

from [13C] 4-maleic anhydride. *Journal of Labelled Compounds and Radiopharmaceuticals* **2014**, 57, (5), 397-401.

25. Rico-Martínez, R.; Snell, T. W.; Shearer, T. L., Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A® to the *Brachionus plicatilis* species complex (Rotifera). *Environmental Pollution* **2013**, 173, 5-10.

26. Anderson, J. A.; Kuhl, A. J.; Anderson, A. N., Toxicity of oil and dispersed oil on juvenile mud crabs, *Rhithropanopeus harrisii*. *Bulletin of environmental contamination and toxicology* **2014**, 92, (4), 375-380.

27. Cohen, J. H.; McCormick, L. R.; Burkhardt, S. M., Effects of dispersant and oil on survival and swimming activity in a marine copepod. *Bulletin of environmental contamination and toxicology* **2014**, 92, (4), 381-387.

28. Stefansson, E. S.; Langdon, C. J.; Pargee, S. M.; Blunt, S. M.; Gage, S. J.; Stubblefield, W. A., Acute effects of non-weathered and weathered crude oil and dispersant associated with the deepwater horizon incident on the development of marine bivalve and echinoderm larvae. *Environmental Toxicology and Chemistry* **2016**.

CHAPTER 3 - SELECTIVE QUANTIFICATION OF DOSS IN MARINE SEDIMENT AND SEDIMENT-TRAP SOLIDS BY LC-QTOF-MS

Matt J. Perkins¹, Samantha B. Joye², Jennifer A. Field^{1*}

¹ Department of Environmental and Molecular Toxicology, Oregon State University,
Corvallis, OR 97331

² Department of Marine Sciences, University of Georgia, Athens, GA 30605

*Corresponding author: jennifer.field@oregonstate.edu; Office: 541 737 2265; Fax: 541
737 0497

Reproduced with permission from Analytical and Bioanalytical Chemistry

3.1 Abstract

At the onset of the 2010 Gulf oil spill, analytical methods for the quantification of the surfactants in Corexit did not exist in the peer-reviewed literature. To date, only a single study reports the presence of DOSS in deep-sea Gulf sediment collected in 2010 from a single location. There are no data on the occurrence of DOSS in association with settling solids (i.e. sediment-trap solids). To address this data gap, DOSS was initially quantified by liquid chromatography tandem quadrupole mass spectrometry (LC-MS/MS) in sediment and sediment-trap solids collected from multiple sites in the Gulf between 2010 and 2013. However, interferences confounded analyses using only a quadrupole (MS/MS) system; therefore, a LC-high mass accuracy quadrupole time of flight mass spectrometry (LC-QTOF-MS) method was developed. The LC-QTOF method was validated and applied to eight representative samples of sediment and of sediment-trap solids. The presented method quantifies DOSS in solids of marine origin at concentrations above the limit of quantification of $0.23 \mu\text{g kg}^{-1}$ with recoveries of $97 \pm 20\%$ (mean $\pm$ 95 CI). Gulf sediment and sediment-trap solids gave DOSS concentrations of $< \text{LOQ}$ - $25 \mu\text{g kg}^{-1}$ and 1.5 - $6.3 \mu\text{g kg}^{-1}$, respectively.

3.2 Introduction

As a response action to the 2010 BP oil spill in the Gulf of Mexico (Gulf), approximately 7 million liters of the chemical dispersants Corexit 9500 and 9527 (hereafter Corexit) were applied to surface-oil slicks in the Gulf and directly at the Deepwater Horizon (DWH) well-head.¹ The purpose of Corexit application was to increase the dispersion of oil in the water column.^{1, 2} Increased dispersion of oil occurs because the surfactant constituents of Corexit reduce the interfacial surface tension of the oil-water interface, promoting the breakup of oil slicks or aggregates into smaller droplets which are then easily dispersed in the water column.²

Bis-(2-ethylhexyl)sulfosuccinate (DOSS) is the anionic surfactant constituent of Corexit (10-21%; mass/mass).³⁻⁵ Corexit also contains the nonionic surfactants Span 80 (2.7-4.4 %), Tween 80 (11-18%) and Tween 85 (4.3-4.6%) as well as other components.⁵ In the water column, the presence of DOSS has been used as a surrogate marker for the polar constituents of Corexit.³ In the absence of degradation, DOSS that remains associated with oil may be used as a surrogate marker for the more hydrophobic constituents of Corexit (e.g. the non-ionic surfactants). It is now well documented that a large fraction of spilled DWH oil underwent sedimentation.^{6, 7} Thus, DOSS may exhibit a large spatial footprint in sediments associated with Gulf seafloor sediments.

Several analytical methods exist for the quantification of DOSS in seawater.^{3-5, 8-10} Yet to date, very little data exists in the peer reviewed literature that characterizes the spatial and temporal trends in DOSS occurrence and concentration in deep-sea Gulf sediments. This situation arose, in part, because validated analytical methods for the extraction and quantification of DOSS in marine sediments did not exist prior to the large-scale application of Corexit during the Gulf oil spill.^{11, 12} The only report of DOSS in Gulf sediments is the study by White et. al. (2014) who reported the presence of DOSS in nine deep-sea sediments from a single site collected in 2010.¹² The study by White was the first literature report of DOSS in Gulf sediments. These authors hypothesized that DOSS applied to surface slicks during the Gulf oil spill emergency-response action underwent an encapsulation event that prevented dissolution, in association with surface

oil, and then subsequently sunk to the seafloor. However, this key paper reported limited analytical performance metrics important to the characterization of DOSS in marine sediments in the Gulf and elsewhere. Additionally, the scope of work did not include validating or applying the analytical method to sedimenting solids (i.e. sediment trap solids) which could contain higher quantities of fresher organic matter relative to sediments. Therefore, sediment-trap solids may present a different matrix requiring separate validation. With this paper, we fill these data gaps by presenting a fully validated analytical methodology to ensure quality data on DOSS occurrence in Gulf solids and report the first evaluation of DOSS in sediment trap solids collected in the Gulf.

3.3 Experimental

3.3.1 Chemicals and Standards. Standards of solid (98.1%) bis-(2-ethylhexyl) sodium sulfosuccinate (DOSS) and deuterated DOSS ($^2\text{H}_{34}$ -DOSS) were obtained from Sigma Aldrich (Saint Louis, MO, USA). A standard of $^{13}\text{C}_4$ -DOSS was provided by Ed Furlong and James Gray of the United States Geological Survey National Water Quality Laboratory (Denver, CO, USA) that had been synthesized by Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Parent stock standards and analytical standards were prepared as described previously (Place et al. 2014).⁵ Analytical standards were prepared in MeOH and analyzed within eight hours. MS-grade methanol (MeOH) was purchased from Fisher Scientific (Pittsburg, PA, USA). Laboratory 18-M Ω , deionized (DI) water was obtained by an in-house Millipore Synergy unit with an LC-Pak polisher (EMD Millipore Corp, Billerica, MA, USA). High purity ammonium acetate was purchased from Sigma Aldrich. Acid-washed sand was purchased from Macron Fine Chemicals (Center Valley, PA, USA). All glassware was combusted at 400 °C for 12 h prior to use.

3.3.2 Sediment and Sediment-Trap Solids Collection. Sediment solids were collected from various sites in the Gulf within 200 km of the DWH wellhead using a multicore device as described by Joye et al. (2010).¹³ Sediment cores were sectioned in 1, 2, 3, or 5 cm intervals, transferred to polypropylene wide mouth jars, frozen at – 20 °C at sea, and shipped to the laboratory on dry ice. Sediment-trap solids were collected at a site within

10 km of the DWH wellhead. Sediment traps were inoculated in situ with mercuric chloride as a biocide, deployed, collected and processed according to Yan et al. (2015).¹⁴ After splitting each cup using a Falcom splitter, 1/8 splits of sediment-trap solids and rinse water were transferred to polypropylene bottles, and shipped to Oregon State University.

3.3.3 Sediment and Sediment-Trap Sample Preparation. The processing of sediment and sediment-trap solids occurred in two phases, 1) preparation for freeze-drying and 2) freeze-drying and mechanical homogenization. During the initial phase each sample was thawed, homogenized by stirring and transferred to multiple polypropylene centrifuge tubes and centrifuged (1140 RCF). The overlying water was removed and the remaining solids (approximately 20-40 g wet weight, WW) were frozen again (-20 °C). The homogenized, frozen samples were stored at -20 °C until being freeze-dried. After being freeze-dried, separate centrifuge tubes containing sub-fractions of the same sediment or sediment-trap sample were combined and mechanically homogenized using a Sorval-Omni-Mixer stick blender (Newtown, CT, USA). Homogenized solids were stored in their original containers, in the dark at room temperature until extraction. Between samples, the stick blender was washed with DOSS-free soap and water; sequentially solvent rinsed with MeOH, isopropanol, acetonitrile, and then MeOH; and then dried under a stream of N₂.

3.3.4 Sediment and Sediment-Trap Solids Extraction. Homogenized sediment (1 - 2 ± 0.1 g) and homogenized sediment-trap solids (0.24 – 1 ± 0.1 g) were placed individually in glass centrifuge tubes, mixed with 3 mL methanol, spiked with 1.4 ng of ¹³C₄ DOSS as a surrogate standard, and sonicated (VWR model number 75HT; Radnor, PA, USA) for 15 min at 40° C. The screw tops of the glass centrifuge tubes were wrapped in baked aluminum foil to prevent contact between the MeOH and the plastic threaded cap. After each sonication cycle, the sample was centrifuged (1140 RCF) for 10 min and 2 mL of extract was removed with a baked glass pasteur pipet and transferred to a fresh baked glass centrifuge tube. The sonication process was repeated with two additional cycles; 2 mL of MeOH was replaced prior to each additional extraction cycle. The three MeOH fractions (6 mL) were combined and blown gently to dryness under N₂ at 55 °C. The dry

residue was spiked with the $^2\text{H}_{34}$ -DOSS internal standard (0.97 ng in 20 μL in MeOH) and reconstituted in 0.5 mL of MeOH via sonication at 40° C, just prior to analysis. All field samples were analyzed within 24 h of extraction and within 8 h of being reconstituted.

3.3.5 Liquid Chromatography. Chromatographic separations were performed on an Agilent 1100 (Agilent Technologies, Inc., Santa Clara, CA, USA) (Place et al. 2014),⁵ with minor modification (see Experimental Methods in Appendix A2.1). Place et al., determined that by adding a C_{18} guard column in the flow path after the purge valve of the LC pump assembly but prior to the autosampler, the retention time of a DOSS contamination peak could be delayed such that baseline resolution was achieved between an injected $^{13}\text{C}_4$ -DOSS peak and the contamination peak, indicating that the LC contained systemic DOSS contamination above the purge valve. Modifications employed included the replacement of this C_{18} guard with two Agilent Zorbax SAX guard columns (4.6 mm ID-12.5 mm length-5 μm particle size). The SAX guards reduced the signal of the systemic DOSS contamination below the limit of detection ($\text{S/N} < 3$) instead of delaying the DOSS contamination originating from within the HPLC, as described by Place et al, (2014). In addition, an external VICI E90 divert valve (Houston, TX, USA) was placed between the analytical column and mass spectrometer. For the analysis, 300 μL of methanolic extract were injected onto an Agilent Poroshell 120 EC- C_{18} guard column (4.6 mm ID x 5 mm length x 2.7 μm particle size) that was placed in-line with an Agilent XDB- C_{18} analytical column (4.6 mm ID x 20 mm length x 3.57 μm particle size). The autosampler was fitted with a 900 μL analytical head and a 900 μL extended seat capillary. The extended seat capillary was necessary to promote the in-line dilution of the 300 μL extract injected and to prevent DOSS from eluting in the void volume of the system. A flow rate program and mobile phase gradient were employed such that DOSS eluted at 4.1 min and the total analysis time was 11.5 min (Appendix A2.1).

3.3.6 Triple Quadrupole Mass Spectrometry (LC-MS/MS). Triple Quadrupole detection was performed on a Waters Micromass Quattro Triple Quadrupole Mass Spectrometer (Waters Corporation; Milford, MA, USA) as described by Place et al., (2014)⁵ and was used for preliminary analyses.

3.3.7 High Resolution Quadrupole Time of Flight (QTOF) Mass Spectrometry.

QTOF analysis was performed on a TripleTOF ABSciex 5600 (Framingham, MA, USA) instrument equipped with an electrospray interface and operated in negative ionization mode at a resolution greater than 20,000 FWHH. In full scan mode, data was collected in 800 cycles with a period cycle time of 450 ms, a pulser frequency of 18.079 kHz, and an accumulation time of 0.1 ms. The curtain, nebulizer (gas 1), and drying (gas 2) gases were 30, 50, and 40 psi, respectively. The ion spray voltage was set at -4500 V with a temperature of 550°C, a collision energy of -10 V, and a declustering potential of -80 V. The starting and ending masses were 70 and 1000, respectively. In QTOF MS/MS mode, the instrument was operated under the same conditions, except with a collision energy of -27 V and spread of 0.0, an ion release delay of 66.63, and an ion release width of 24.92. The instrument was calibrated with the APCI Negative Calibration Solution (ABSciex) before each extract was injected.

The instrument was run concurrently in full scan mode to capture the molecular ions of DOSS (m/z 421.2265), $^{13}\text{C}_4$ -DOSS (m/z 425.2400 $\pm$ 15 ppm) and $^2\text{H}_{34}$ -DOSS (m/z 455.4400) and in QTOF mode to collect MS/MS fragment data for DOSS and its isotopes. Percent isotopic deviation and exact mass agreement were determined using Masterview (version 2.2). Quantification for LC-QTOF-MS was performed using Multiquant (version 2.1). Molecular ions for DOSS (m/z 421.2265 $\pm$ 15 ppm), the $^{13}\text{C}_4$ -DOSS surrogate standard (m/z 425.2400 $\pm$ 15 ppm) and the $^2\text{H}_{34}$ -DOSS internal standard (m/z 455.4400 $\pm$ 15 ppm) were extracted for quantification. The fragment ions of DOSS (m/z 227.1278 and 80.9156 $\pm$ 15 ppm), $^{13}\text{C}_4$ -DOSS (m/z 231.1423 and 80.9156 $\pm$ 15 ppm) and $^2\text{H}_{34}$ -DOSS (m/z 244.2356 and 80.9156 $\pm$ 15 ppm) were used for confirmation.

3.3.8 LC-QTOF-MS Calibration. Separate calibration curves for DOSS and $^{13}\text{C}_4$ -DOSS were prepared on each day of analysis and each consisted of five calibration standards that spanned from 0.27 to 3.6 ng injected DOSS or $^{13}\text{C}_4$ -DOSS. The $^2\text{H}_{34}$ -DOSS (internal standard) was added to all calibration standards, such that 0.97 ng of $^2\text{H}_{34}$ -DOSS were injected. The calculated concentration of each calibration standard was required to be within 20% of its nominal value to be used for quantification. Calibration curves were 1/x weighted and required to give a linear correlation coefficient of 0.99 or greater. The

reported concentration of DOSS was calculated from calibration curves using the $^2\text{H}_{34}$ -DOSS internal standard. The percent recovery of the $^{13}\text{C}_4$ -DOSS surrogate standard was calculated from separate calibration curves using the $^2\text{H}_{34}$ -DOSS internal standard. As a result, the reported DOSS concentrations are not corrected for surrogate recovery. One extract gave DOSS at a concentration above the upper limit of the calibration range. This sample was re-extracted and reanalyzed with a five point calibration curve spanning from 0.27 to 28 ng of injected DOSS.

3.3.9 LC-QTOF-MS Quality Control. Check standards were used for quality control purposes and consisted of 900 ng L^{-1} so that 0.27 ng DOSS was injected. Check standards were run every eight samples, were required to have a calculated concentration within 20% of the expected value, and were used to verify the continued performance of the instrument. To continually check for DOSS contamination, blank MeOH and an extraction blank were run at least every eight samples along with the check standards. Blank MeOH samples were required to give signals below the instrument limit of detection ($s/n < 3$) and demonstrated that no carryover or instrument contamination was present. Extraction-process blanks were used to identify DOSS signals resulting from the extraction process. Extraction-process blanks consisted of taking 1 g acid-washed and combusted (400°C , 12 h) sand through the same extraction process described for field samples. Extraction-process blanks ($n = 18$), gave quantifiable DOSS signals ranging from $s/n < 3$ to an equivalent of $0\text{--}0.61 \mu\text{g kg}^{-1}$ (Table A2.1). The DOSS signals originated from the extraction process and were used to define the whole method lower limit of quantification (see lower limit of quantification section).

3.3.10 Effects of Sample Processing and Storage. The potential for DOSS to be lost during sample processing was determined by 1) analyzing the overlying water that was removed from sediments during centrifugation and 2) by comparing the concentration of DOSS in sediment that was freeze-dried to sediment that was not freeze-dried. During the first phase of laboratory sample processing (see Sediment and Sediment-Trap Sample Preparation section), a small fraction of mass (3-4 g WW) was removed from the total mass (20-40 g WW) of a select number of samples for the purposes of evaluating the concentration of DOSS with and without freeze-drying. These sub-samples were stored at

-20 °C until they were extracted in triplicate using the same methods used for freeze-dried sediment except that 1.0 ± 0.1 g WW sediment was extracted and not ~1g DW. The analysis of the overlying water was performed using LC-MS/MS as described by Place et al.⁵ The overlying water was < LOD with respect to DOSS. The initial analysis of DOSS in wet sediment ($n = 3$) was completed using LC-MS/MS and a single sediment was identified containing environmentally incurred DOSS. Interferences were not observed in the chromatograms of DOSS, $^{13}\text{C}_4$ -DOSS and $^2\text{H}_{34}$ -DOSS. The concentration of environmentally-incurred DOSS in this wet sediment was compared on a dry weight basis to sediment from the same sample that was freeze-dried ($n = 4$). No significant difference (Student's t-test, $\alpha = 0.05$) was observed between sediments that were freeze-dried and those that were not.

The storage stability of DOSS in freeze-dried sediments was evaluated by comparing the concentration of DOSS calculated for separate extracts ($n = 4$) of a single sediment to the concentration of DOSS determined 12 months later. The concentration of DOSS was evaluated for significant difference at the 95% confidence interval. No significant difference in concentration was observed over 12 months. Freeze dried sediments were stored at room temperature in the dark. Sediments that were not freeze dried were stored at -20° C in the dark.

3.3.11 LC-QTOF-MS Whole Method Accuracy and Precision. Freeze-dried and homogenized Gulf sediment (25 g), previously determined to be free of DOSS, was spiked with 25 ng of DOSS to yield a final sediment concentration of $1 \mu\text{g kg}^{-1}$. The spiked sediment was dried in the dark at room temperature for four days and then re-homogenized. Four separate aliquots of this spiked sediment were then extracted and analyzed by LC-QTOF-MS. Accuracy was defined as the ratio of the calculated analyte concentration to the spiked analyte concentration.

A robust whole method error term was calculated that incorporates within run and between day reproducibility associated with extracting and analyzing separate aliquots of a single sediment from the Gulf containing environmentally incurred DOSS. Four separate aliquots (1 ± 0.1 g) of this single sediment were extracted and then the extracts were analyzed on three separate days for a total of 12 analyses. The whole method error

term reported in table 1 was calculated as the relative standard deviation of these 12 analyses.

3.3.12 Lower Limit of Quantification (LOQ). Because extraction blanks gave positive signals for DOSS ($s/n < 3 - 0.61 \mu\text{g kg}^{-1}$), they were used to define the whole method LOQ. The upper limit of the 99.9% confidence interval about the computed mean DOSS concentration ($0.09 \mu\text{g kg}^{-1}$) in extraction blanks was used to define the whole method LOQ ($0.23 \mu\text{g kg}^{-1}$). For this reason, no limit of detection was determined.

3.4 Results and Discussion

3.4.1 Extract Cleanup. Methanolic extracts were generated using methods¹¹ similar to those reported by White et al.¹² and analyzed for DOSS using LC-MS/MS. Extensive investigations into off-line and on-line extract cleanup techniques, including cleanup based on various solid phase extraction media (e.g., strong anion exchange, C_{18} , and silica), varying the polarity of the extraction solvent, and chromatographic separation were conducted. These cleanup techniques were investigated in an attempt to resolve interferences that were observed in the selected reaction monitoring chromatograms for the nominal-mass-to-charge transition for DOSS ($m/z\ 421 \rightarrow 81$), $^{13}\text{C}_4$ -DOSS ($m/z\ 425 \rightarrow 81$), and $^2\text{H}_{34}$ -DOSS ($m/z\ 455 \rightarrow 81$) and compromised the analysis by LC-MS/MS (Figure 1). The degree of these interferences varied between extracts of sediments collected from different sites in the Gulf and between sediment-trap solids. Extract cleanup proved ineffective in removing matrix interferences, presumably because the causative agents partition similarly to DOSS. Because no clean-up of extracts was possible, high mass accuracy LC-QTOF-MS was pursued because the selectivity of LC-QTOF-MS can reduce interferences resulting from complex sample matrices.¹⁵

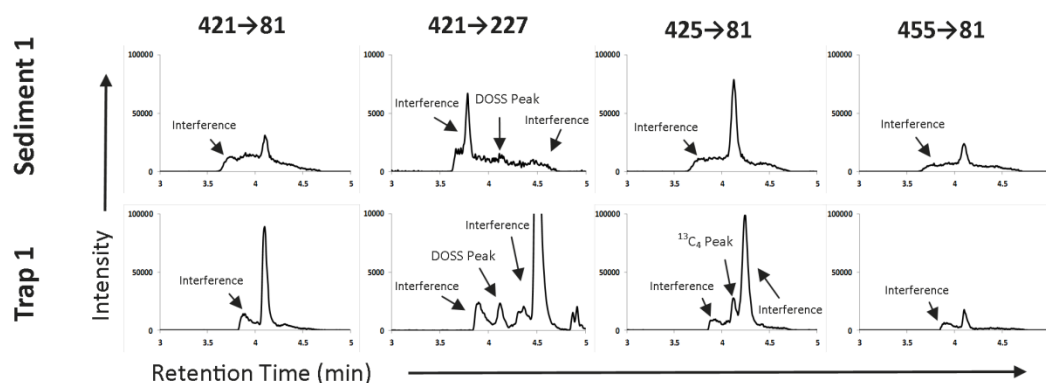


Figure 3.1. Interferences in the selected reaction monitoring chromatograms for the nominal-mass-to-charge transitions for DOSS (m/z 421→81 and m/z 421→227), $^{13}\text{C}_4$ -DOSS (m/z 425→81), and $^2\text{H}_{34}$ -DOSS (m/z 455→81) in a sediment and sediment-trap extract analyzed by LC-MS/MS

3.4.2 LC-QTOF-MS Optimization. The high mass accuracy LC-QTOF-MS chromatograms of the molecular ions of DOSS (m/z 421.2265 $\pm$ 15 ppm), $^{13}\text{C}_4$ -DOSS (m/z 425.2400 $\pm$ 15 ppm) and $^2\text{H}_{34}$ -DOSS (m/z 455.4400 $\pm$ 15 ppm) were free of interferences without the need for extract cleanup (Figure 2). Therefore, accuracy, precision and the instrumental LOQ of the LC-QTOF-MS analysis were determined.

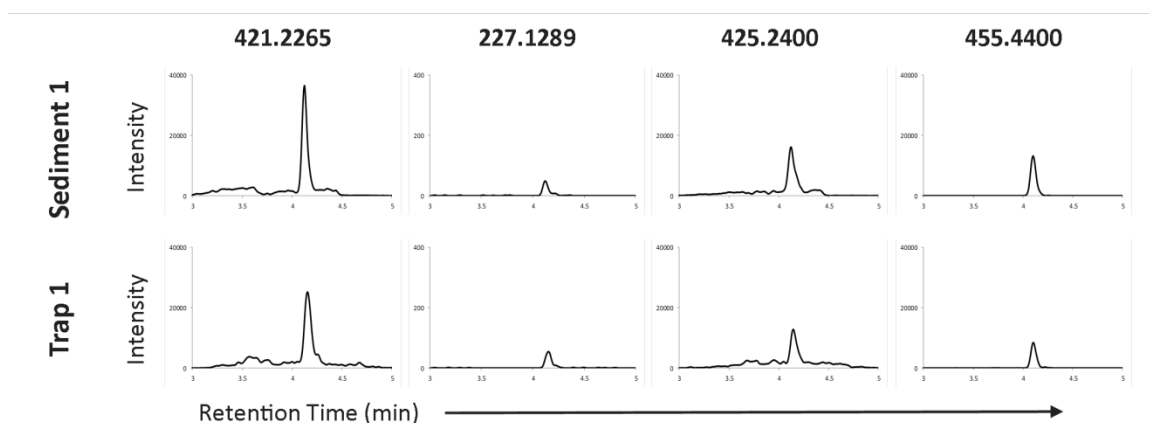


Figure 3.2. Typical extracted ion chromatograms of the molecular ions of DOSS (m/z 421.2265 ± 15 ppm), $^{13}\text{C}_4$ -DOSS (m/z 425.2400 ± 15 ppm), and $^2\text{H}_{34}$ -DOSS (m/z 455.4400 ± 15 ppm), and the confirmation fragment ion of DOSS (m/z 227.1278 ± 15 ppm) analyzed by LC-QTOF-MS

Whole method accuracy, as determined by the absolute recoveries of spiked DOSS and the $^{13}\text{C}_4$ -DOSS surrogate standard in blank Gulf sediment, were 97 ± 20 and $88 \pm 16\%$, respectively (Table 1). When DOSS was ratioed to the $^{13}\text{C}_4$ -DOSS surrogate standard, the relative recovery increased to $120 \pm 9\%$ (Table 1). Only one other study (White et al.) exists reporting the extraction of DOSS in marine sediments. The whole method accuracy for DOSS is similar to that reported by White et al. (2014). They measured DOSS recovery between 89 and 119% using $^2\text{H}_{34}$ -DOSS as an internal standard. No surrogate standard was used in the method of White et al., preventing comparison. Precision (RSD), determined from spike and recovery experiments ($n = 4$), was 13% and 12% for DOSS and $^{13}\text{C}_4$ -DOSS, respectively (Table 1). The LOQ for DOSS was $0.23 \mu\text{g kg}^{-1}$ (Table 1). The only other literature report of the extraction of DOSS from sediments of marine origin (White et al., SSSS2014), did not report an LOQ, thus preventing comparison. Combusted-sand blanks (process blanks; $n = 18$), gave quantifiable DOSS signals that originated from the extraction process and ranged from $s/n < 3$ to $0.61 \mu\text{g kg}^{-1}$ (Table S1). White et al. (2014) also analyzed combusted-sand blanks and observed DOSS concentrations ranging from 0 - $0.99 \mu\text{g kg}^{-1}$.

Table 3.1. Absolute and relative accuracy (average recovery $\pm$ 95% CI) and precision (% RSD) of spiked DOSS ($1.0 \mu\text{g kg}^{-1}$) and spiked $^{13}\text{C}_4$ -DOSS ($1.4 \mu\text{g kg}^{-1}$) onto a blank Gulf of Mexico sediment and the instrumental LOQ for DOSS determined from extraction blanks.

	LC-QTOF-MS (n=4)
DOSS absolute recovery (%)	97 ± 20 (95% CI) (13% RSD)
$^{13}\text{C}_4$ -DOSS absolute recovery (%)	88 ± 16 (95% CI) (12% RSD)
DOSS recovery relative to $^{13}\text{C}_4$ -DOSS (%)	120 ± 9 (95% CI) (5% RSD)
Instrument DOSS LLOQ $\mu\text{g kg}^{-1}$)	0.23
Between day precision (RSD) Sediment Core 7 (n = 12)	20 %

3.4.3 Effects of sample processing and storage. Sample processing consisted of centrifugation, removal of the overlying water, and freeze-drying of the remaining solids. The potential for DOSS to be lost during sample processing was evaluated by 1) identifying a single Gulf sediment that contained environmentally incurred DOSS, 2) analyzing the overlying water for DOSS and 3) comparing the concentration of DOSS for subsamples that were either freeze-dried or not freeze-dried. The overlying water was $<$ LOD with respect to DOSS. No significant difference at the 95% CI in the DOSS concentration was observed for sub-samples that were freeze-dried relative to those that were not (Table A2.2).

Sample stability was evaluated by analyzing separate extracts of the same freeze-dried sediment 12 months after the initial analysis. No significant difference in the concentration of DOSS at the 95% CI was observed over 12 months (Table A3.2)

3.4.4 LC-QTOF-MS Method Demonstration. The LC-QTOF-MS method was applied to representative sediment (n = 8) and sediment-trap solids (n = 8) collected from the

Gulf to demonstrate the method. Observed DOSS in sediments ranged from below the LOQ to $25 \mu\text{g kg}^{-1}$ DW (Table 2). White et al., reported concentrations of DOSS ranging from $19\text{--}9000 \mu\text{g kg}^{-1}$ dry weight for deep sea sediments collected at a single site in the Gulf in 2010. The highest concentration of DOSS observed in sediments in this study is several orders of magnitude below the highest concentration reported by White et al. However, this difference could be explained by the extreme spatial heterogeneity in the distribution of DOSS in deep-sea sediments reported by White et al. The percent recovery of the ^{13}C -DOSS surrogate standard in sediments ranged from 84.8 to 113% (Table 2). The extraction efficiency of the spiked internal standard is expected to be higher than that of the incurred environmental residue of DOSS in aged sediments.^{16, 17} Therefore recovery of the ^{13}C -DOSS surrogate standard indicates quantitative transfer of DOSS through the extraction process rather than exhaustive extraction of the environmentally incurred residue of DOSS. However, during method development, the extractable fraction of incurred DOSS residues in aged sediment was extracted within 3 sequential extractions.

The validated high mass accuracy LC-QTOF-MS method was also applied to the extracts of eight sediment-trap solids. Sediment-trap solids exhibited concentrations of DOSS ranging from 1.5 to $6.3 \mu\text{g kg}^{-1}$ (Table 2). The percent recovery of the ^{13}C -DOSS surrogate standard in sediment-trap solids ranged from 83.8 to 111% (Table 2). Concentrations of DOSS in sediment-trap solids have yet to be reported in the literature, thus, preventing comparison of these values to others.

Table3. 2. Concentration of DOSS in extracts of marine sediment and sediment-trap solids, recovery of ^{13}C -DOSS, DOSS exact mass agreement (ppm), DOSS isotopic agreement (%), and latitude and longitude reported in degrees decimal minutes. Uncertainties about the reported DOSS concentrations are $\pm 13\%$ (see Table 1).

Sample Number	DOSS ($\mu\text{g kg}^{-1}$)	Percent recovery $^{13}\text{C}_4$ -DOSS	DOSS exact mass agreement (ppm)	DOSS isotopic distribution agreement (%)	Latitude N	Longitude W
Sediment 1	<LOD	84.8	NC	NC	27° 21.946'	90° 33.847'
Sediment 2	<LOD	104	NC	NC	28° 51.12'	89° 3.47'
Sediment 3	0.82	91.2	0.1	<10	28° 32.95'	88° 40.76'
Sediment 4	2.5	87.3	0	<10	28° 34.21'	88° 19.41'
Sediment 5	1.7	109	-0.2	<10	28° 32.20'	87° 56.38'
Sediment e 6	0.81	91.4	-0.1	<10	28° 41.989'	88° 30.122'
Sediment 7	1.15	92.5	7.7	<20	28° 40.78'	88° 21.68'
Sediment 8	25	113	3.1	<10	29° 53.48'	88° 24.95'
Trap 1	2.2	111	0.8	<20	28° 42.55'	88° 25.34'
Trap 2	1.5	99.8	-0.2	<10	28° 42.55'	88° 25.34'
Trap 3	4.0	84.0	0.3	<10	28° 42.55'	88° 25.34'
Trap 4	3.8	111	2.3	<10	28° 42.55'	88° 25.34'
Trap 5	1.5	83.8	0.5	<10	28° 42.55'	88° 25.34'
Trap 6	2.2	108	-0.1	<10	28° 42.55'	88° 25.34'
Trap 7	1.4	101	-0.6	<10	28° 42.55'	88° 25.34'
Trap 8	6.3	89.4	-1.9	<10	28° 42.55'	88° 25.34'

< LOQ less than the lower limit of quantification (0.23 $\mu\text{g/kg}$)

NC not computed because DOSS < LOQ

The high mass accuracy capability of the ABSCIEX 5600 QTOF provides additional selectivity for DOSS by confirming the exact mass and isotopic distribution of the molecular ions and fragment ions of interest. The exact mass error of DOSS in sediment and sediment-trap extracts was less than 5 parts per million (ppm) for all but one extract, which was below 10 ppm (Table 2). The characteristic fragment ions of DOSS (m/z 80.9156 and m/z 227.1289) were observed within expected abundance ratios for all sediment and sediment-trap extracts indicating selectivity for DOSS. The isotopic distribution deviated by less than 10% of the theoretical distribution for all but one extract, which deviated by less than 20% of the theoretical distribution (Table 2). The combination of exact mass agreement for DOSS and its characteristic fragment ions, isotopic distribution agreement, and agreement of fragment ion abundance ratios with retention time matched standards, represents the highest tier of confidence for small compound identification.¹⁸

3.4.5 Whole method evaluation of matrix effects. As expected, matrix effects inhibited the ionization of target analytes as indicated by a reduction in the area counts of the $^2\text{H}_{34}$ -DOSS internal standard observed in extracts of sediment (17-77% decrease, mean = 45%) and sediment-trap solids (54-93% decrease, mean = 76%) relative to a solvent matrix. Ionization suppression was higher in sediment-trap solids compared to sediments, possibly due to the greater quantity and freshness of organic material. The sensitivity of the LC-QTOF was sufficient ($s/n > 10$) to allow for integration of the $^2\text{H}_{34}$ -DOSS internal standard in all samples analyzed indicating sufficient ionization of the target analytes in all sediment extracts analyzed. Reproducible recoveries of the DOSS in blank Gulf sediment spike and recovery samples ($97 \pm 20\%$, Table 2) and the ^{13}C -DOSS surrogate standard in all extracts analyzed (83.8-111 %, Table 2) indicate that the use of an isotopically labeled internal standard was sufficient to compensate for the matrix effects encountered in this study.⁴ Additionally, the use of an isotopically labelled analog of the target analyte is a well-established technique for compensating for matrix effects without sacrificing accuracy or reproducibility.¹⁹⁻²¹

3.5. Conclusions

Interferences confounded the initial attempts to analyze sediment and sediment-trap solids by LC-MS/MS. In contrast, LC-QTOF-MS was more selective for DOSS by providing confirmation of the exact mass and isotopic distribution of the molecular ion and fragment ions of interest. Thus, we recommend the use of high mass accuracy mass analyzers for future analyses of DOSS in complex sediment and sediment-trap solids. The analysis of sediments presented in this study confirms the presence of DOSS in deep-sea sediments in the Gulf originally reported by White et al. (2014). Future work will include the quantification of DOSS in a larger set of sediments collected in the Gulf from 2010 through 2015 and sediment-trap solids collected in the Gulf in 2012 allowing the elucidation of the spatial and temporal trends of DOSS occurrence after the 2010 Gulf oil spill.

3.6 Literature Cited

1. National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling. The Use of Surface and Subsea Dispersants During the BP Deepwater Horizon Oil Spill. **2011**.
2. National Research Council. Dispersant Oil Spill Efficacy and Effects. **2005**.
3. Kujawinski, E. B.; Soule, M. C. K.; Valentine, D. L.; Boysen, A. K.; Longnecker, K.; Redmond, M. C., Fate of Dispersants Associated with the Deepwater Horizon Oil Spill. *Environ Sci Technol* **2011**, *45*, (4), 1298-1306.
4. Ramirez, C. E.; Batchu, S. R.; Gardinali, P. R., High sensitivity liquid chromatography tandem mass spectrometric methods for the analysis of dioctyl sulfosuccinate in different stages of an oil spill response monitoring effort. *Anal Bioanal Chem* **2013**, *405*, (12), 4167-4175.
5. Place, B. J.; Perkins, M. J.; Sinclair, E.; Barsamian, A. L.; Blakemore, P. R.; Field, J. A., Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. *Deep Sea Research Part II: Topical Studies in Oceanography* **2014**.
6. Valentine, D. L.; Fisher, G. B.; Bagby, S. C.; Nelson, R. K.; Reddy, C. M.; Sylva, S. P.; Woo, M. A., Fallout plume of submerged oil from Deepwater Horizon. *Proceedings of the National Academy of Sciences* **2014**, *111*, (45), 15906-15911.
7. Ziervogel, K.; Joye, S. B.; Arnosti, C., Microbial enzymatic activity and secondary production in sediments affected by the sedimentation pulse following the Deepwater Horizon oil spill. *Deep Sea Research Part II: Topical Studies in Oceanography* **2014**.
8. Mathew, J.; Schroeder, D. L.; Zintek, L. B.; Schupp, C. R.; Kosempa, M. G.; Zachary, A. M.; Schupp, G. C.; Wesolowski, D. J., Dioctyl sulfosuccinate analysis in near-shore Gulf of Mexico water by direct-injection liquid chromatography-tandem mass spectrometry. *J Chromatogr A* **2012**, *1231*, 46-51.

9. Gray, J. L.; Kanagy, L. K.; Furlong, E. T.; Kanagy, C. J.; McCoy, J. W.; Mason, A.; Lauenstein, G., Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. **2014**, 95, 124-130.
10. Parker, A. M.; Ferrer, I.; Thurman, E. M.; Rosario-Ortiz, F. L.; Linden, K. G., Determination of COREXIT components used in the Deepwater Horizon cleanup by liquid chromatography-ion trap mass spectrometry. *Anal Methods-Uk* **2014**, 6, (15), 5498-5502.
11. Perkins, M., Place, B.J., Field, J., Analysis of DOSS and its degradates by LVI-LC/ESI-MS/MS. *Gulf of Mexico Oil Spill and Ecosystem Science Conference* **2013**, New Orleans, Jan 21-23.
12. White, H. K.; Lyons, S. L.; Harrison, S. J.; Findley, D. M.; Liu, Y.; Kujawinski, E. B., Long-Term Persistence of Dispersants following the Deepwater Horizon Oil Spill. *Environ Sci Technol Lett* **2014**, 1, (7), 295-299.
13. Joye, S. B.; Bowles, M. W.; Samarkin, V. A.; Hunter, K. S.; Niemann, H., Biogeochemical signatures and microbial activity of different cold-seep habitats along the Gulf of Mexico deep slope. *Deep Sea Research Part II: Topical Studies in Oceanography* **2010**, 57, (21), 1990-2001.
14. Yan, B., Passow, U, Chanton, J3, Nöthig, EM Asper, V, Sweet, J. Pitiranggon, M, Diercks, A, Pak, D., Sustained deposition of contaminants from the Deepwater Horizon spill. *PNAS* **2015**, *In Press*.
15. Sancho, J. V.; Pozo, Ó. J.; Ibáñez, M.; Hernández, F., Potential of liquid chromatography/time-of-flight mass spectrometry for the determination of pesticides and transformation products in water. *Anal Bioanal Chem* **2006**, 386, (4), 987-997.
16. Burford, M. D.; Hawthorne, S. B.; Miller, D. J., Extraction rates of spiked versus native PAHs from heterogeneous environmental samples using supercritical fluid extraction and sonication in methylene chloride. *Analytical Chemistry* **1993**, 65, (11), 1497-1505.

17. Hatzinger, P. B.; Alexander, M., Effect of aging of chemicals in soil on their biodegradability and extractability. *Environ Sci Technol* **1995**, 29, (2), 537-545.
18. Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J., Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ Sci Technol* **2014**, 48, (4), 2097-2098.
19. Trufelli, H.; Palma, P.; Famiglini, G.; Cappiello, A., An overview of matrix effects in liquid chromatography–mass spectrometry. *Mass spectrometry reviews* **2011**, 30, (3), 491-509.
20. Gosetti, F.; Mazzucco, E.; Zampieri, D.; Gennaro, M. C., Signal suppression/enhancement in high-performance liquid chromatography tandem mass spectrometry. *J Chromatogr A* **2010**, 1217, (25), 3929-3937.
21. Annesley, T. M., Ion suppression in mass spectrometry. *Clinical chemistry* **2003**, 49, (7), 1041-1044.

CHAPTER 4 - SPATIAL AND TEMPORAL TRENDS IN DOSS IN GULF OF MEXICO DEEP-SEA SEDIMENTS: 2010 AND 2015

Matt J. Perkins¹, Alix E. Robel¹, Samantha B. Joye², Jennifer A. Field¹

¹ Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR 97331

² Department of Marine Sciences, University of Georgia, Athens, GA 30605

*Corresponding author: jennifer.field@oregonstate.edu; Office: 541 737 2265; Fax: 541 737 0497

In preparation for submission

4.1 ABSTRACT

During the Deepwater Horizon (DWH) oil spill of 2010, approximately 7 million L of Corexit (9527A/9500A) were applied in the Gulf of Mexico (Gulf). Dioctyl Sulfosuccinate (DOSS) is the anionic surfactant constituent of Corexit. DOSS was quantified in the top layer (0-5 cm) of 39 sediment cores collected between 2010 and 2015. The spatial distribution of 2010 and 2011 DOSS impacted sediments observed in the present study is discussed in the context of publicly-available but unpublished data on DOSS in sediments under the Natural Resource Damage Assessment (NRDA) program to define the foot print of DOSS in surficial sediments through 2011. Positive observations of DOSS ranged from 0.82 to 6100 $\mu\text{g kg}^{-1}$ and the spatial footprint of DOSS-impacted sediments through 2011 lies primarily within 10 km of the wellhead and extends outside of this radius ~100 km to the NE, ~130 km to the N and ~150 km to the SW of the DWH. The temporal trends observed in sediments collected in the present study between 2010 and 2015 are discussed. DOSS was observed at concentrations ranging from 0.74-2.3 $\mu\text{g kg}^{-1}$ within 13 km of the DWH wellhead through 2015 demonstrating the long term persistence of DOSS in deep-sea sediments in the Gulf.

4.2 Introduction

During the Emergency response to the British Petroleum oil spill of 2010, the oil-dispersant Corexit (9500A and 9527A), was applied to surface slicks (3.1 million L) and directly at the wellhead (2.9 million L) to increase the dispersion of oil into the water column.¹ Corexit increases the dispersion of oil by reducing the interfacial-surface tension of the oil-water interface, promoting the formation of smaller oil droplets than would form in the absence of Corexit.² However, the use of chemical dispersant represents an ecological tradeoff and the full environmental ramifications of the use of Corexit during the oil spill are still unfolding.³⁻⁵ Therefore, it is essential that the transport and persistence of Corexit in the marine environment be fully documented to aid decision making regarding the use of Corexit during future emergency oil spill response actions.

Although Corexit is a complex mixture of carrier solvent and surfactants², only some of the constituents of Corexit were monitored in the aftermath of the emergency oil spill response.⁶⁻¹¹ Most literature reports of Corexit in the Gulf of Mexico (Gulf) have focused on the water column and on a single component of Corexit, DOSS.^{6, 8-11} Only a single report(White et al.) documents DOSS in deep-sea Gulf sediments.¹² This key study was the first to report the presence of DOSS in deep sediments of the Gulf, yet it provided only limited information on the spatial distribution (n = 9) of DOSS in deep-sea sediments collected in 2010.

To address the potential long-term persistence of DOSS in the deep sediments of the Gulf, DOSS was quantified in 39 sediment cores collected by Ecosystem Impacts of Oil and Gas into the Gulf (ECOGIG) between 2010 and 2015. The spatial and temporal distribution of DOSS in these sediments is discussed and compared with publically-available DOSS concentrations in sediment that were reported as part of the National Resource Damage Assessment (NRDA) process.

4.3 Experimental

4.3.1 Chemicals and Standards. All analytical standards and stable isotopes used for the analysis are described in Appendix A3.1. Information regarding reagents and standard preparation protocols is provided in Appendix A3.1.

4.3.2 Sediment Collection. Sediment cores were collected between 2010 and 2015 within 350 km of the DWH wellhead using a multicore device as described by Joye et al. (2010) (Figure 1).¹³ Sediment cores were treated as described in Perkins et al.

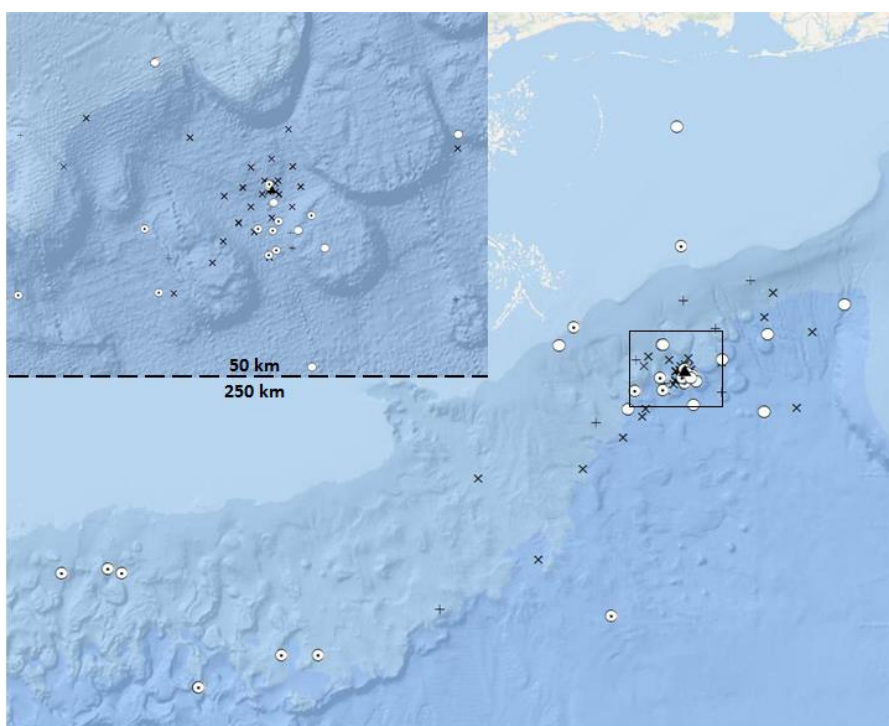


Figure 4.1. Spatial distribution of 39 sediment cores collected between 2010 to 2011 (white circles) and 2012 to 2015 (white circle with black dot), 18 sediment cores collected as part of the NRDA process on the Holiday Chouest cruise (+), and 114 sediment cores collected as part of NRDA on the Sarah Bordelon cruise (x). The black triangle represents the DWH. The inlay in the upper left represents an enlargement of the area near the DWH surrounded by the black square

4.3.3 Sediment Processing and Sediment Extraction. The processing of sediments is described in detail in Perkins et al., 2016. Briefly, sediment materials were centrifuged,

freeze-dried, extracted with methanol at 40° C, and the final extract was spiked with $^2\text{H}_{34}$ -DOSS. Preliminary data indicated that DOSS was only in quantifiable concentrations in the top most layer (0-1, 0-3, or 0-5 cm) of each sediment core so only the top most layer of each sediment core was analyzed for DOSS.

4.3.4 Liquid Chromatography (LC) High Resolution Quadrupole Time of Flight (QTOF) Mass Spectrometry. Chromatographic separations were performed on an Agilent 1100 (Agilent Technologies, Inc., Santa Clara, CA, USA) that was coupled to a TripleTOF ABSciex 5600 (Framingham, MA, USA) mass spectrometer as described by Perkins et al. and in Appendix A3.2.

4.3.5 NRDA Dataset. The NRDA program collected data on DOSS concentrations in sediments collected on the Holiday Chouest and Sarah Bordelon cruises. Sediments were collected in 2011 by push core ($n = 18$) on the Holiday Chouest cruise and by multicore ($n = 114$) on the Sarah Bordelon cruise (Figure 1). Information on how to locate this data set is provided in Appendix A3.3.

4.4 Results and Discussion.

A limited number of sediments were collected by ECOGIG between 2010 and 2015. However, the 2011 NRDA samples represent a larger dataset on sediment DOSS concentrations. Therefore, the spatial distribution of DOSS in sediments collected by ECOGIG in 2010 and 2011 is discussed in the context of the 2011 NRDA dataset to define the spatial distribution of DOSS-impacted sediments. The temporal trend in DOSS is then inferred from DOSS concentrations in sediments collected between 2010 and 2015.

4.4.1 Spatial Trend in DOSS Concentrations: 2010-2011. DOSS was observed in 55% of the surficial sediments collected by ECOGIG in 2010 and 2011 at concentrations ranging from 0.81 to 25 $\mu\text{g kg}^{-1}$ (dry weight, DW) (Table 1) All positive observations of DOSS in 2010 and 2011 surficial sediments were within 130 km of the DWH (Table 1). Sediments that did not contain DOSS are listed in Appendix Table A3.1. No correlation

between sediment DOSS concentrations and radial distance from the DWH was observed for sediments collected by ECOGIG in 2010 and 2011 (Figure 2).

Table 4.1. ECOGIG data reported by year collected, DOSS concentration ($\mu\text{g kg}^{-1}$), radial distance from the DWH (km), latitude and longitude (decimal degrees), and the depth interval (cm) for surficial sediments observed to contain DOSS contamination. Uncertainties about the reported DOSS concentrations are $\pm 13\%$.

Year	DOSS Concentration ($\mu\text{g kg}^{-1}$)	Radial Distance (km)	Latitude	Longitude	Depth (cm)
2010	18	1.5	28.7243	-88.3645	0-5
2010	2.5	19	28.57017	-88.3235	0-1
2010	17	20	28.78803	-88.167	0-3
2010	0.82	37	28.54917	-88.67933333	0-2
2010	1.7	49	28.53667	-87.93966667	0-3
2010	25	130	29.89133	-88.41583333	0-1
2011	1.2	5.3	28.69833	-88.33833333	0-3
2012	1.2	0.39	28.73933	-88.36966667	0-3
2012	1.8	0.47	28.7413	-88.36911667	0-3
2012	2.1	6.5	28.67967	-88.36133333	0-3
2012	0.81	13	28.69982	-88.50203333	0-3
2015	2.3	3.5	28.70683	-88.359	OF
2015	0.74	4.5	28.69783	-88.36516667	0-5

In the 2011 NRDA dataset, DOSS was observed in 11% and 92% of surficial sediments collected on the Holiday Chouest (Table A3.2) and Sarah Bordelon (Table A3.3) cruises at concentrations ranging from $< \text{LLOQ} - 9.3 \mu\text{g kg}^{-1}$ and $< \text{LLOQ} - 6100 \mu\text{g kg}^{-1}$, respectively. Positive observations of DOSS in sediments collected on the Holiday Chouest cruise ($6.1-9.3 \mu\text{g kg}^{-1}$, mean = 7.7) (Table A3.2) are in agreement with the range of DOSS concentrations observed in 2010 sediments in the present study (Table 1).

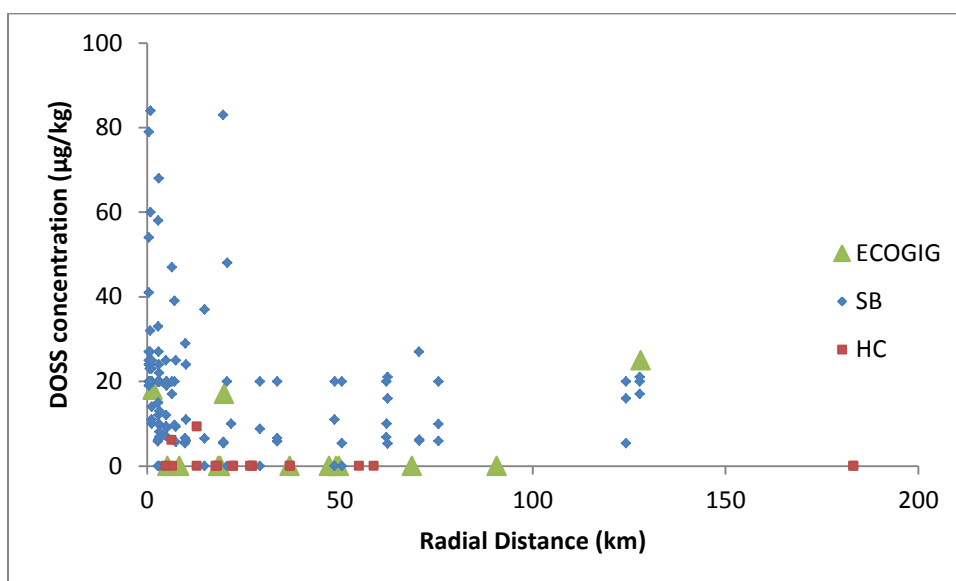


Figure 4.2. DOSS sediment concentrations ($\mu\text{g kg}^{-1}$) collected by ECOGIG (green triangle), the Holiday Chouest (NRDA) cruise (red square), and Sarah Bordelon (NRDA) cruise (blue diamond) in sediments plotted against radial distance from the DWH (km) in 2010 and 2011. Zero represents the LLOQ ($0.23 \mu\text{g kg}^{-1}$ for this study and the $5 \mu\text{g kg}^{-1}$ for NRDA data).

In contrast, two populations of DOSS-impacted sediments are apparent in the Sarah Bordelon dataset. DOSS was observed in one surficial sediment collected within 1 km of the DWH at a concentration of $6100 \mu\text{g kg}^{-1}$, whereas, the majority (99%) of positive observations of DOSS are characterized by lower concentrations ($5.3\text{--}84 \mu\text{g kg}^{-1}$, mean = 20). The low DOSS concentrations associated with Sarah Bordelon sediments are in much closer agreement with the ECOGIG and Holiday Chouest datasets.

White et al. collected 9 sediment cores from 2 sites within 22 km of the DWH in 2010 and also observed two populations of DOSS impacted surficial sediments: sediments impacted by low DOSS concentrations ($n = 2$, $19\text{--}22 \mu\text{g kg}^{-1}$) and high DOSS concentrations ($n = 3$, $2400\text{--}9000 \mu\text{g kg}^{-1}$). Therefore, the observations of the ECOGIG and NRDA datasets are consistent with the observations of White et al. Combined, these three datasets indicate that Gulf sediments are primarily characterized by DOSS

concentrations in the tens of $\mu\text{g kg}^{-1}$ surrounding a much smaller footprint of highly-impacted sediments in the thousands of $\mu\text{g kg}^{-1}$. This spatial distribution of DOSS-impacted sediments lies primarily within 10 km of the wellhead and extends outside of this radius primarily to the N (~ 130 km), NE (~ 100 km), and SW (~ 150 km).

No strong correlation between surficial sediment DOSS concentration and radial distance from the DWH is observed for Sarah Bordelon sediments (Figure 2). A small but significant decrease in the mean DOSS concentration is observed for sediments collected between 10 and 20 km (19 ± 3.9 , mean $\pm$ 95 CI) from the DWH relative to those collected within 10 km (23 ± 4.0 , mean $\pm$ 95 CI). However, this statistically-significant decrease may be explained by limited sampling at distances greater than 10 km that was insufficient to capture DOSS heterogeneously deposited on the seafloor. In contrast, sediment markers of spilled Macondo oil associated with deep-water plume fallout (i.e. point source) have been documented to be strongly correlated with radial distance from the DWH.^{14, 15} Therefore, these data are consistent with the hypothesis of White et al: the application of DOSS to surface slicks during the Gulf oil spill emergency-response action and the subsequent sinking of DOSS-oil-flocculent material to the seafloor. In particular, the observation of DOSS in sediment collected on the continental shelf 130 km north of the DWH (Table 1 row 6) is difficult to rationalize as having originated from a source other than the surface application of Corexit. Plume-derived DOSS would likely have been impinged on the continental shelf prior to reaching this location.^{14, 16, 17}

4.4.2 Temporal trends of DOSS impacted sediments 2010-2015. DOSS occurred at concentrations greater than $5 \mu\text{g kg}^{-1}$ through 2011 within ~ 150 km of the DWH (Table 1, Table A3.2 and Table A3.3). In contrast, in 2012 and 2015, DOSS concentrations in sediments collected within 13 km of the DWH ranged from 0.74 to $2.3 \mu\text{g kg}^{-1}$. DOSS was not observed in sediments collected in 2013 and sediments were not acquired in 2014 by ECOGIG.

DOSS was observed in sediments at a lower frequency, at lower concentrations, and at shorter radial distances in sediments collected between 2012 and 2015 compared to those collected in 2010 and 2011. However, fewer sediments were collected from 2012-2015, which prohibits a statistical comparison between the 2012 and 2015

sediments to the 2010 and 2011 sediments. Although it is difficult to determine if DOSS are significantly declining, the continued occurrence of DOSS in sediments in 2015 indicates the persistence of DOSS in deep-sea sediments more than 5 years following the application of Corexit during the 2010 Gulf oil spill. DOSS biodegrades under aerobic conditions^{3,9, 18} of the water column but no data documents the degradation of DOSS under anaerobic/anoxic conditions.

4.5 Implications. The spatial footprint of DOSS-impacted sediments within 150 km of the DWH represents a 14,000 km² area characterized by DOSS concentrations ranging between ~1 and 84 µg kg⁻¹. A smaller area within 20 km of DWH is characterized by higher (>2400 µg kg⁻¹) DOSS concentrations. DOSS exposure to benthic dwelling organisms likely occurred and impact to benthic organisms resulting from the DWH oil spill is documented.¹⁹⁻²¹ Data on the occurrence of DOSS in sediments collected 5 years after the original application of Corexit indicates long-term persistence of DOSS in sediments compared to its rapid dissipation from the water column.

4.6 Literature Cited

1. National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling. The Use of Surface and Subsea Dispersants During the BP Deepwater Horizon Oil Spill. 2011.
2. National Research Council. Dispersant Oil Spill Efficacy and Effects. 2005.
3. Kleindienst S, Seidel M, Ziervogel K, Grim S, Loftis K, Harrison S, et al. Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. *Proceedings of the National Academy of Sciences*. 2015;201507380.
4. Almeda R, Hyatt C, Buskey EJ. Toxicity of dispersant Corexit 9500A and crude oil to marine microzooplankton. *Ecotoxicology and environmental safety*. 2014;106:76-85.
5. DeLeo DM, Ruiz-Ramos DV, Baums IB, Cordes EE. Response of deep-water corals to oil and chemical dispersant exposure. *Deep Sea Research Part II: Topical Studies in Oceanography*. 2016;129:137-47.
6. Hayworth JS, Clement TP. Provenance of Corexit-related chemical constituents found in nearshore and inland Gulf Coast waters. *Marine pollution bulletin*. 2012;64(10):2005-14.
7. Parker AM, Ferrer I, Thurman EM, Rosario-Ortiz FL, Linden KG. Determination of COREXIT components used in the Deepwater Horizon cleanup by liquid chromatography-ion trap mass spectrometry. *Anal Methods-Uk*. 2014;6(15):5498-502.
8. Place BJ, Perkins MJ, Sinclair E, Barsamian AL, Blakemore PR, Field JA. Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. *Deep Sea Research Part II: Topical Studies in Oceanography*. 2014.

9. Kujawinski EB, Soule MCK, Valentine DL, Boysen AK, Longnecker K, Redmond MC. Fate of Dispersants Associated with the Deepwater Horizon Oil Spill. *Environ Sci Technol*. 2011;45(4):1298-306.
10. Mathew J, Schroeder DL, Zintek LB, Schupp CR, Kosempa MG, Zachary AM, et al. Dioctyl sulfosuccinate analysis in near-shore Gulf of Mexico water by direct-injection liquid chromatography-tandem mass spectrometry. *J Chromatogr A*. 2012;1231:46-51.
11. Gray JL, Kanagy LK, Furlong ET, Kanagy CJ, McCoy JW, Mason A, et al. Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. 2014;95:124-30.
12. White HK, Lyons SL, Harrison SJ, Findley DM, Liu Y, Kujawinski EB. Long-Term Persistence of Dispersants following the Deepwater Horizon Oil Spill. *Environ Sci Technol Lett*. 2014;1(7):295-9.
13. Joye SB, Bowles MW, Samarkin VA, Hunter KS, Niemann H. Biogeochemical signatures and microbial activity of different cold-seep habitats along the Gulf of Mexico deep slope. *Deep Sea Research Part II: Topical Studies in Oceanography*. 2010;57(21):1990-2001.
14. Valentine DL, Fisher GB, Bagby SC, Nelson RK, Reddy CM, Sylva SP, et al. Fallout plume of submerged oil from Deepwater Horizon. *Proceedings of the National Academy of Sciences*. 2014;111(45):15906-11.
15. Stout SA, Payne JR. Macondo oil in deep-sea sediments: Part 1—sub-sea weathering of oil deposited on the seafloor. *Marine Pollution Bulletin*. 2016.
16. Romero IC, Schwing PT, Brooks GR, Larson RA, Hastings DW, Ellis G, et al. Hydrocarbons in deep-sea sediments following the 2010 Deepwater Horizon blowout in the northeast Gulf of Mexico. *Plos One*. 2015;10(5):e0128371.
17. Beyer J, Trannum HC, Bakke T, Hodson PV, Collier TK. Environmental effects of the Deepwater Horizon oil spill: a review. *Marine Pollution Bulletin*. 2016.

18. Campo P, Venosa AD, Suidan MT. Biodegradability of Corexit 9500 and dispersed South Louisiana crude oil at 5 and 25 C. *Environ Sci Technol*. 2013;47(4):1960-7.
19. White HK, Hsing P-Y, Cho W, Shank TM, Cordes EE, Quattrini AM, et al. Impact of the Deepwater Horizon oil spill on a deep-water coral community in the Gulf of Mexico. *Proceedings of the National Academy of Sciences*. 2012;109(50):20303-8.
20. Montagna PA, Baguley JG, Cooksey C, Hartwell I, Hyde LJ, Hyland JL, et al. Deep-sea benthic footprint of the Deepwater Horizon blowout. *Plos One*. 2013;8(8):e70540.
21. Fisher CR, Hsing P-Y, Kaiser CL, Yoerger DR, Roberts HH, Shedd WW, et al. Footprint of Deepwater Horizon blowout impact to deep-water coral communities. *Proceedings of the National Academy of Sciences*. 2014;111(32):11744-9.

**CHAPTER 5 - EJECTION OF SURFACTANTS FROM COREXIT
9500A/9527A AND ALKANES FROM OIL INTO THE ATMOSPHERE VIA
AEROSOLIZATION FROM THE SEA SURFACE**

Paria Avij^{*,1}, Matt J. Perkins^{*2}, He Huang¹, Francisco R. Hung¹, Jennifer A. Field²,
Kalliat T. Valsaraj¹

¹ Cain Department of Chemical Engineering, Louisiana State University, Baton
Rouge, LA, USA

² Department of Environmental and Molecular Toxicology, Oregon State University,
Corvallis, OR, USA

*Denotes that these authors made equal contributions

Submitted to Environmental Processes and Impacts

In response to reviewer comments

5.1 Abstract.

Corexit was used extensively during the 2010 Deepwater Horizon (DWH) oil spill, in part, to disperse surface-slick oil into the water column. This study quantifies the ejection rate of surfactants from Corexit 9500A/9527A, as well as alkanes from oil, as they are aerosolized into the atmosphere via bursting bubbles in a laboratory bubble column reactor. Oil was premixed with Corexit 9500A or 9527A at a dispersant-to oil ratio (DOR) of 1:20 and injected at two different rates into a laboratory bubble column reactor filled with either NaCl solution or seawater. Aerosol samples taken at the top of the column show important quantities of the Corexit surfactants DOSS, Tween 80 and Tween 85, with larger ejection rates observed when the column is filled with seawater as compared to NaCl solution. Additionally, for both seawater and NaCl solution, premixing dispersants with oil was found to enhance the ejection rates of intermediate, semi-volatile, and non-volatile organic compounds (IVOCs/SVOCs/NVOCs) from oil, as compared to the situation where oil without dispersant is directly injected into the column. On the other hand, the ejection rate of volatile organic compounds (VOCs) was found to be comparable for the case where standalone oil and oil premixed with Corexit 9500A were injected into the column; however, significantly lower ejection rates of VOCs were observed when oil premixed with Corexit 9527A was injected. Our results provide compelling evidence that aerosolization via bursting bubbles at the sea surface can be an important transport vector for oil spill matter (namely surfactants from dispersants and alkanes from oil) into the atmosphere.

5.2 Introduction

Crude oil is released into oceanic waters via natural seepage and through human activities such as oil production, oil transport, and offshore oil exploration.¹ During the Deepwater Horizon (DWH) oil spill, the largest marine oil spill in U.S. history, an approximate amount of 4.9 million barrels of crude oil were released into the Gulf of Mexico (GOM).^{1, 2} The amount of hydrocarbon released over 3 months was 40-170 times greater than the annual total natural seepage of oil in the GOM (4000-17000 tons, as estimated from satellite remote sensing techniques).³⁻⁵

Oil dispersants are mixtures of solvents and surfactants that are used as one of the ways to combat off-shore oil spills. The objectives of applying dispersants are to reduce oil delivery to shoreline ecosystems and to increase oil dissolution into the water column, presumably making it more bioavailable.^{6, 7} During the DWH oil spill, about 1.8 million gallons of the dispersants, Corexit 9527A and 9500A, were applied at the water surface 1.1 million gallon and in-situ at the well-head (0.7 million gallon).^{8, 9} Existing stocks of Corexit 9527A were used first, and after they were exhausted, Corexit 9500A was used to disperse the spilled oil.⁹ The main difference between these two formulations is the presence of 2-butoxyethanol as a solvent in Corexit 9527A, which is not a component of Corexit 9500A due to toxicity concerns.¹⁰ Both formulations of Corexit are designed to break up the oil into small droplets (<70 μm in diameter) by reducing the oil-water interfacial tension.¹¹

The environmental fate of the surfactant components of Corexit and their potential ecological trade-offs are still under study.¹²⁻¹⁵ Since 2010, the occurrence of Corexit surfactants in the water column^{9, 16, 17} and Gulf of Mexico sediments¹⁸⁻²⁰ have been documented, and several studies have focused on processes that affect the fate of the primary anionic surfactant in Corexit, dioctyl sulfosuccinate (DOSS), e.g., biodegradation,²¹ photolysis,²² and sorption onto sediments.²³

Application of Corexit is also credited for the reduction in volatile organic air concentrations, which may have reduced worker exposure to volatiles, ultimately enabling a more rapid response to the oil spill.²⁴ Gaseous and aerosol composition

measurements during the DWH oil spill were conducted by the National Oceanic and Atmospheric Administration (NOAA) using WP-3D aircraft and ships in the vicinity of the DWH spill site.²⁵ The presence of wide aerosol plumes downwind of DWH spill site was attributed to the formation of Secondary Organic Aerosol (SOA) from several oil precursors, namely intermediate and semi-volatile organic compounds (IVOCs and SVOCs).²⁶ The possibility of the direct ejection of oil matter through sea sprays was ruled out due to the observed increase in the particle size distribution downwind.²⁶ However, the ratio of hydrocarbon-like organic aerosol (HOA) to oxygenated organic aerosol (OOA) downwind of the spill site exhibited higher levels compared to those measured at the spill site. This observation is in contradiction to the hypothesis of attributing the formation of aerosol plume to purely oxidative SOA formation. In our previous studies combining lab experiments²⁷ and molecular simulations²⁸, we demonstrated for the first time that significant quantities of long chain alkanes (C15-C30) from oil can be ejected into the atmosphere via bursting bubbles at the sea surface, even though some of these oil alkanes might have limited volatility. Aerosol droplets produced by bursting bubbles during wave breaking are considered the main source of sea sprays and one of the major contributors to the particulate matter flux into the atmosphere.²⁹⁻³² As discussed by Ehrenhauser et al.²⁷, the continuous aerosolization of less volatile oil matter downwind of the DWH spill site can explain the higher HOA to OOA ratio mentioned above. Our studies demonstrated for the first time that aerosolization via bursting bubbles can be an important transport vector for oil spill matter into the atmosphere.

Building on our previous studies^{27, 28} here we report laboratory experiments with the objective of determining if the surfactant components of Corexit 9500A/9527A could be aerosolized into the atmosphere via bursting bubbles. We focused our analysis on the most abundant Corexit surfactants DOSS (10-21%; mass/mass), Tween 80 and Tween 85 (15.3-22.6%; mass/mass).^{16, 33, 34} Evaporation of these surfactants into the atmosphere is not expected given their very low vapor pressure. In our previous lab experiments²⁷ we did not evaluate the ejection of these surfactants. The adsorption of organic, natural-derived, and anthropogenic (e.g., perfluorooctanoate) surfactants at the

air-water interface of bubbles and their ejection into the atmosphere through the breaking of bubbles has been documented.³⁵⁻³⁸ However, to the best of our knowledge, the possible aerosolization of Corexit surfactants has not been evaluated before. Furthermore, in our previous experiments²⁷ we used a dispersant-to-oil ratio (DOR) of 1:100, which is significantly different from the DOR of 1:20 reportedly used during the DWH oil spill.⁸ In our earlier experiments we also used a solution of sodium chloride in deionized water instead of seawater, for simplicity and to directly link our experiments with our modeling results.²⁸

In our current study, in addition to measuring the emission rate of surfactants we also report additional measurements of the ejection of oil alkanes, using a DOR of 1:20 and considering both NaCl solution and seawater from the Gulf of Mexico. Using actual seawater allowed us to assess the effect of ions other than Na⁺ and Cl⁻ (e.g., Ca²⁺, Mg²⁺, SO₄²⁻), as well as organic and surface-active materials, on the ejection rates of components from dispersants and oil.

The rest of our paper is structured as follows. Details of our experiments (materials and standards, setup and operation of our aerosolization reactor, analysis of surfactants and alkanes, and determination of ejection rates) are presented in Section 2. Section 3 contains our results and discussion, and our conclusions are summarized in Section 4.

5.3 Experimental

5.3.1 Materials and Standards. The crude oil used for experiments was supplied by the BP Gulf Coast Restoration Organization (Houston, TX, USA). Corexit 9527A and Corexit 9500A were obtained from Nalco Environmental Solutions, LLC (Sugar Land, TX, USA) and they were used as received.²⁷ Seawater was collected from the Gulf of Mexico near Destin, Florida. Alkane standards and internal standards for alkane analysis methyl decanoate, methyl arachidate, methyl octacosanoate were obtained from Sigma Aldrich (St Louis, Mo, USA).²⁷ Ethyl acetate (HPLC grade), used as a solvent for alkanes, was purchased from EMD Millipore (Billerica, MA, USA). Standards of solid (98.1%) bis-(2-ethylhexyl) sodium sulfosuccinate (DOSS) was obtained from Sigma

Aldrich (Saint Louis, MO, USA). A standard of 13C4–DOSS was provided by Ed Furlong and James Gray of the United States Geological Survey National Water Quality Laboratory (Denver, CO, USA) that had been synthesized by Cambridge Isotope Laboratories, Inc (Andover, MA, USA). MS-grade methanol and isopropanol, used for surfactant analysis, were purchased from Fisher Scientific (Pittsburg, PA, USA). Laboratory 18-M Ω , deionized (DI) water was obtained by an in-house Millipore Synergy unit with an LC-Pak polisher (EMD Millipore Corp, Billerica, MA, USA). High purity ammonium acetate was purchased from Sigma Aldrich. All glassware was baked at 400 °C for 12 h prior to use.

5.3.2 Laboratory Aerosolization Experiments. All experiments were conducted in a bubble column reactor (Figure A3.1) developed in Valsaraj's laboratory and described by Smith et al.³⁹ The aerosol generation by bursting bubbles was adapted from Keene et al.⁴⁰ and was tuned in our previous work to study the aerosolization of oil/dispersant matter (Bubble flow 5 L min⁻¹, shear flow 6.4 L min⁻¹, and air-lift flow 40 L min⁻¹).²⁷ Greater detail is provided in Appendix A4.1. Throughout the experiments, either oil or a mixture of oil and Corexit (9500A or 9527A) at the dispersant-to-oil ratio of 1:20 (1.6 mL dispersants premixed with 32 mL crude oil), was injected into the reactor. The reactor was filled with NaCl solution or seawater. Oil or an oil-dispersant mixture was injected in separate experiments at 20 and 50 μ L min⁻¹ using a syringe pump (KD scientific Model # 210, Holliston, MA, USA).

When the reactor reached steady state, the effluent of the reactor was collected by two sampling methods: (1) constant mass-flow air-sampling nozzle, which allows collection of both vapors and particles; and (2) electrostatic precipitator (ESP), which only allows particle collection. For nozzle collection, the effluent of the reactor was sampled by a constant mass-flow air-sampling nozzle in a bubbler that was filled with ethyl acetate (alkane analysis) or with isopropanol (surfactant analysis). The ethyl acetate and isopropanol were analyzed directly by GC-MS/GC-FID and LC-MS/MS, respectively. For ESP collection, particulates were captured on an aluminum target as described in Ehrenhauser et al.²⁷ The aluminum target was then extracted in ethyl acetate or isopropanol and the solvent was then analyzed directly by GC-MS/GC-FID and LC-

MS/MS, respectively. For both nozzle and ESP collection, duplicate samples were collected over a time period of 15 min 1 m above the water surface. For both nozzle and ESP collection, blanks were collected as described previously, except that no oil nor surfactant was injected into the bubble column.

5.3.3 LC-MS/MS for Corexit Surfactant Analysis. All samples for the analysis of the surfactant constituents of Corexit were shipped overnight on dry ice to Oregon State University and stored at -20° C until analysis. Quantification of DOSS, Tween 80 and Tween 85 was performed using an Agilent 1100 (Agilent Technologies, Inc., Santa Clara, CA, USA) interfaced with a Waters Acquity Triple Quadrupole Mass Spectrometer or a Waters Micromass Quattro Mass Spectrometer (Waters Corporation, Milford, MA, USA), as described in Place et al., with minor modification (Appendix A4.2). Isopropanol samples were diluted by 75% with Instant Ocean® salt mix solution (Spectrum Brands Company, Madison, WI, USA) to meet existing analytical methods.

5.3.4 GC-MS and GC-FID for Alkane Analysis. Ethyl acetate, collected by nozzle and ESP, was dried over sodium sulfate and analyzed by GC-MS and GC-FID to procure their alkane concentrations and the subsequent ejection rates of alkanes into the air. Quantification of alkanes by GC-MS and GC-FID are described in our previous study by Ehrenhauser et al.²⁷ and in Appendix A4.3. During GC-MS and GC-FID analysis, peak areas found in the blank samples were subtracted from peak areas found in the experimental samples. The net peak area was then used to calculate concentrations of alkanes in the experimental samples.

5.3.5 Calculation of Ejection Rates. The concentrations of surfactants and alkanes were converted to an ejection rate. The ejection rates of the Corexit surfactants are reported differently from those of the alkanes because the surfactants showed particularly large variability in the rate of ejection at different reactor injection rates (i.e., when injected into the reactor at 20 and 50 $\mu\text{L min}^{-1}$). The ejection rates of DOSS, Tween 80 and Tween 85 are reported as the mean of duplicate samples for the separate injection rates of 20 and 50 $\mu\text{L min}^{-1}$. Tween 80 and Tween 85 are reported as the combined contribution of both Tween 80 and Tween 85 (i.e., Tween 80 + Tween 85).

The ejection rates of alkanes are reported as a normalized ejection rate for VOCs, IVOCs, SVOCs and NVOCs. Similar trends in the ejection rates of alkanes were observed when injected into the reactor at 20 and 50 $\mu\text{L min}^{-1}$. For example, the ejection rate of NVOCs from NaCl increased by 59 and 87 percent relative to the oil-only control when the Corexit 9500A-oil mixture was injected into the reactor at 20 and 50 $\mu\text{L min}^{-1}$. Therefore, the ejection rates of alkanes were first normalized to a percentage of the oil-only control. Then the normalized ejection rates calculated for a reactor injection rate of 20 and 50 $\mu\text{L min}^{-1}$ were pooled. The normalized ejection rates of alkanes are reported as the mean $\pm$ 95% CI.

5.3.6 Salinity, Surface Activity, and Organic Carbon Content of Seawater.

Experiments were conducted in seawater from the GOM to study the possible effects of the inorganic and organic constituents of seawater on the ejection of oil and dispersant components by bursting bubbles. The salt concentration of the seawater was measured by conductivity meter and through comparison with standard solutions of known salt concentrations.⁴¹ The seawater surface tension was measured using a Kruss tensiometer (K14/141, Hamburg, Germany). The total organic carbon of seawater and NaCl solution were measured by Shimadzu Total Organic Carbon (TOC) Analyzer (EPA method #415.1).

5.4 Results and Discussion.

5.4.1 The Ejection of Corexit Surfactants Detected by Nozzle Collection (NaCl Solution). The ejection of DOSS, Tween 80 and Tween 85 from NaCl solution via bursting bubbles was detected when the reactor effluent was sampled by nozzle collection (Table 1), indicating that DOSS, Tween 80 and Tween 85 can be aerosolized by bursting bubbles. The ejection of Corexit surfactants by bursting bubbles is a novel and previously uncharacterized transport vector. Nozzle collection captures materials ejected in both the vapor and particulate phases. However, DOSS is anionic and Tween 80 and Tween 85 are complex mixtures of oligomers with average molecular weights greater than 1000 g

mol-1.17, 42 Therefore, DOSS, Tweens 80 and Tween 85 detected by nozzle collection are expected to have been ejected in the form of particulate matter.

The rate of ejection of DOSS from NaCl solution was 0.08 and 0.29 $\mu\text{g min}^{-1}$ when Corexit 9527A, premixed with crude oil, was injected into the bubble column at 20 and 50 $\mu\text{L min}^{-1}$, respectively. (Table 1). The rate of ejection of Tween 80 and Tween 85 was below the limit of quantification (LOQ) and 0.05 $\mu\text{g min}^{-1}$ when the Corexit 9527A-oil mixture was injected at 20 and 50 $\mu\text{L min}^{-1}$, respectively (Table 1). The ratio of DOSS to Tween 80 and Tween 85 was not calculated when the Corexit 9527A-oil mixture was injected at 20 $\mu\text{L min}^{-1}$ because Tween 80 and Tween 85 were below the LOQ (Table 1). However, when Corexit 9527A was injected into the reactor at 50 $\mu\text{L min}^{-1}$, the ratio of DOSS to Tween 80 and Tween 85 was 6.0. The ratio of DOSS to Tween 80 and Tween 85 in the commercial formulation of Corexit 9527A is approximately 0.8.17 The greater than seven fold increase in the ratio of DOSS to Tweens in the atmospheric particle phase may suggest that DOSS, in the presence of a crude oil and Corexit 9527A mixture, is more readily adsorbed at the air-water interface relative to the Tweens.

Similar rates of ejection of DOSS, Tween 80 and Tween 85 are expected between Corexit 9527A and 9500A because the mass percent contribution of these individual surfactants is very similar between the two formulations.¹⁷ When Corexit 9500A was premixed with crude oil and injected into the bubble column at 20 and 50 $\mu\text{L min}^{-1}$, the rate of ejection of DOSS was 0.10 and 0.34 $\mu\text{g min}^{-1}$ (Table 1), which is similar to the rate observed for Corexit 9527A. However, the rate of ejection of Tween 80 and Tween 85 from the Corexit 9500A-oil mixture was below the LOQ when injected at 20 and 50 $\mu\text{L min}^{-1}$ (Table 1). This may suggest greater variability in the ejection rates of Tween 80 and Tween 85 from NaCl solution relative to DOSS. The ratio of DOSS to Tween 80 and Tween 85 was not calculated for Corexit 9500A detected by nozzle collection because Tween 80 and Tween 85 were below the LOQ (Table 1).

Table 5.1. Average rate of ejection ($\mu\text{g min}^{-1}$) of DOSS, Tween 80 and Tween 85 from NaCl solution and seawater. Oil was premixed with Corexit 9527A or 9500A and injected in to the reactor at 20 and 50 $\mu\text{L min}^{-1}$ and emitted particles were collected by nozzle and ESP collection. Data are reported as the mean of duplicate data points.

	Nozzle			ESP		
	Corexit Surfactant Ejection Rate (µg min-1)					
Experiment	DOSS	ΣTweens	DOSS/ΣTweens	DOSS	ΣTweens	DOSS/ΣTweens
Corexit 9527A (NaCl)						
Blank	<LOQ	<LOQ	NC	<LOQ	<LOQ	NC
20 µL min-1	0.08	<LOQ	NC	0.13	0.04	3.0
50 µL min-1	0.29	0.05	6.0	0.32	0.21	1.5
Corexit 9500A (NaCl)						
Blank	<LOQ	<LOQ	NC	<LOQ	<LOQ	NC
20 µL min-1	0.10	<LOQ	NC	0.07	0.01*	7.0
50 µL min-1	0.34	<LOQ	NC	0.06	0.01	5.5
Corexit 9500A (Seawater)						
Blank	<LOQ	<LOQ	NC	<LOQ	<LOQ	NC
20 µL min-1	2.2	0.05	45	1.4	0.12	12
50 µL min-1	3.4	0.08	43	1.8	0.06	31

*= Only a single data point because one duplicate was <LOQ

NC=Not calculated *

* The ratio of DOSS to Tween 80 and Tween 85 was not calculated because the concentration of Tween 80 and Tween 85 in aerosol phase were below the LOD

Table 5.2. Normalized ejection rate ($\mu\text{g min}^{-1}$) (mean $\pm$ 95 CI) of VOCs (C10-C14), IVOCs (15-C19), SVOCs (C20-C24) and NVOCs (C25-C29) collected by nozzle and ESP. Alkane ejection rates in the presence of Corexit 9527A or 9500A are normalized to the ejection rate of alkanes in the oil-only control (no dispersant present).

	Nozzle				ESP			
Experiment	C10-C14	C15-C19	C20-C24	C25-C29	C10-C14	C15-C19	C20-C24	C25-C29
Corexit 9527 (NaCl)	0.86 $\pm$ 0.04	1.3 $\pm$ 0.15	1.7 $\pm$ 0.30	1.6 $\pm$ 0.43	<LOQ	5.9 $\pm$ 2.1	1.9 $\pm$ 0.62	2.6 $\pm$ 0.45
Corexit 9500 (NaCl)	1.0 $\pm$ 0.09	1.6 $\pm$ 0.23	2.4 $\pm$ 1.2	1.7 $\pm$ 0.32	<LOQ	4.5 $\pm$ 2.3	2.1 $\pm$ 0.50	2.3 $\pm$ 0.53
Corexit 9500 (Seawater)	0.99 $\pm$ 0.22	1.3 $\pm$ 0.09	2.0 $\pm$ 0.82	6.4 $\pm$ 2.9	<LOQ	2.0 $\pm$ 0.4	1.5 $\pm$ 0.54	1.7 $\pm$ 0.43

5.4.2 The Ejection of Corexit Surfactants Detected by ESP Collection (NaCl

Solution). The ejection of DOSS, Tween 80 and Tween 85 from NaCl solution was also detected when the reactor effluent was sampled by ESP collection (Table 1), confirming the ejection of DOSS, Tween 80 and Tween 85 associated with particulate matter via bursting bubbles. The rate of ejection of DOSS was 0.13 and 0.32 $\mu\text{g min}^{-1}$ when Corexit 9527A, premixed with crude oil, was injected into the bubble column at 20 and 50 $\mu\text{L min}^{-1}$, respectively (Table 1). These rates of ejection of DOSS observed by ESP collection are similar to those observed for Corexit 9527A collected by nozzle, suggesting that the rate of ejection of DOSS is highly reproducible. However, this reproducibility was not observed for Corexit 9500A collected by ESP. When the Corexit 9500A-oil mixture was injected into the bubble column at 20 and 50 $\mu\text{L min}^{-1}$, the rate of ejection of DOSS was 0.07 and 0.06 $\mu\text{g min}^{-1}$, respectively (Table 1). This rate of ejection of DOSS was more than 4 fold less than all other observations (Table 1).

The rate of ejection of Tween 80 and Tween 85 was 0.04 and 0.21 $\mu\text{g min}^{-1}$ when the Corexit 9527A-oil mixture was injected at 20 and 50 $\mu\text{L min}^{-1}$ and detected by ESP, respectively (Table 1). For the Corexit 9500A-oil mixture, however, the rate of ejection of the Tweens was 0.01 $\mu\text{g min}^{-1}$ at an injection rate of 20 and 50 $\mu\text{L min}^{-1}$ (Table 1). Variability in the rate of ejection of the Tweens continued to be high when the reactor effluent was collected by ESP. However, the ratio of DOSS to Tweens was consistently greater than 0.8 (Table 1), supporting the observations that DOSS is more readily available for transport to the atmosphere via bursting bubbles relative to Tween 80 and Tween 85.

Some of the variability observed in the collection of surfactant particles by ESP may be due to the chemical composition and surface active nature of surfactant particles, which could influence the collection efficiency of ESP.⁴³ The low resistant (highly conductive) nature of surfactants can impede the effective and reproducible collection of these particles on the collecting electrode of ESP since a weak electric field is maintained across the collected layer of conductive particles.⁴⁴ In spite of the variability in the rate of ejection of surfactants in different treatments, both ESP and nozzle show the ejection of surfactant components in the same order of magnitude.

5.4.3 The Effect of Seawater on the Ejection of Corexit Surfactants. Much higher rates of ejection (> 4 fold) were observed for DOSS from seawater than from NaCl solution (Table 1). The rate of ejection of DOSS from seawater ranged from 1.4 to 2.2 $\mu\text{g min}^{-1}$ when collected by nozzle and ESP (Table 1). These differences in the rate of ejection of DOSS observed for NaCl solution and seawater may be the result of the presence of natural surfactants, organic materials and other salts in seawater. The seawater surface tension (67 mN/m) was lower than the NaCl solution (72 mN/m), indicating the presence of natural surfactants in seawater. The presence of these materials may increase the partitioning of DOSS to the air-water interface, ultimately increasing the ejection of DOSS into the atmosphere.^{45, 46} Alternatively, the different rates of ejection could be the result of differences in the ionic strength ($\Delta 0.06 \text{ M}$) or TOC ($\Delta 6.4 \text{ mg L}^{-1}$) between the seawater and NaCl solution. These observations suggest that the rate of ejection of DOSS via bursting bubbles in the environment with salinity of approximately 35 ‰ (w/w) will be greater than those observed for NaCl solutions.

The rate of ejection of Tween 80 and Tween 85 from seawater was variable, ranging from 0.05 to 0.12 $\mu\text{g min}^{-1}$ when collected by nozzle and ESP (Table 1), confirming the observations for NaCl solution. However, the ratio of DOSS to Tween 80 and Tween 85 was 10-50 fold greater than the ratio present in the original formulation (Table 1), further supporting the observation that DOSS is more readily available for transport to the atmosphere via bursting bubbles relative to Tween 80 and Tween 85.

5.4.4 The Effect of Corexit on the Rate of Ejection of VOCs from NaCl Solution. The application of Corexit 9527A had a small but significant, at the 95 percent confidence interval decrease (14%) in the normalized rate of ejection for VOCs (C10-C14) from NaCl solution relative to the oil only control (Table 2). This may indicate that Corexit 9527A increases the mass of VOCs retained in the water column, reducing the mass of VOCs at the air-water interface available to evaporate to the atmosphere. However, no significant difference in the normalized rate of ejection of VOCs from NaCl solution was observed in the presence of Corexit 9500A relative to the oil only control (Table 2).

The results of this study contrast with those of Ehrenhauser et al,²⁷ where Corexit 9500A was applied at the DOR of 1:100 and the rates of ejection of VOCs increased relative to the oil only control. This may suggest that increasing the application of Corexit to a DOR of 1:20 increases the effectiveness of surfactants in retaining volatile alkanes in the water column, limiting their rates of ejection relative to oil only control. When the reactor effluent was collected by ESP, the VOCs were below the LOQ (Table 2). VOCs are primarily ejected into the air in the form of vapors rather than particulates. The inability to detect VOCs by ESP collection was expected and confirms that ESP collection captures particulate matter but not volatiles.

5.4.5 The Effect of Corexit on the Rate of Ejection of Volatile and Particulate IVOCs, SVOCs and NVOCs from NaCl Solution. The ejection of the combined volatile and particulate fractions of alkanes (“combined fractions”; i.e., nozzle collection) into the atmosphere above the bubble column was greatest for IVOCs (C15-C19) followed by SVOCs (C20-C24) and NVOCs (C25-C29) for Corexit 9527A and 9500A (Table S1). The ejection of the combined volatile and particulate fractions of alkanes ranged from 7.7-14, 0.28-0.96 and 0.28-0.63 $\mu\text{g min}^{-1}$ for IVOCs, SVOCs, and NVOCs, respectively (Table S1).

The presence of Corexit 9527A resulted in a significant increase in the normalized rate of ejection of the combined fractions of alkanes for IVOCs, SVOCs, and NVOCs from NaCl solution in comparison to the oil only control (Table 2). The mean increase in the normalized rate of ejection of alkanes relative to the oil only treatment was 30%, 70%, and 60% for IVOCs, SVOCs, and NVOCs, respectively (Table 2). Corexit 9500A also had a significant increase at the 95 percent confidence interval in the normalized rate of ejection of the combined fractions of alkane IVOCs, SVOCs, and NVOCs from NaCl solution relative to oil only control when collected by nozzle (Table 2). The mean increase in the normalized rate of ejection was 60%, 140%, and 70% for IVOCs, SVOCs, and NVOCs, respectively (Table 2). There was no significant difference in the normalized rate of ejection of IVOCs, SVOCs, and NVOCs observed between Corexit formulations (Table 2). The similar effect of both Corexit formulations on the ejection of the combined fractions of IVOCs, SVOCs, and NVOCs may indicate that differences in

formulation^{17, 42} did not significantly change the surface-driven transport of oil matter via bursting bubbles.

The enhancement in the rates of ejection of IVOCs, SVOCs, and NVOCs in the presence of Corexit can be attributed to the large density of bubbles produced inside the reactor by the same volume of air in the presence of dispersant.²⁵ Corexit decreases the surface tension of the aqueous phase which allows for a greater number of smaller air bubbles to be produced from a given volume of air than would be produced in the absence of Corexit. The resulting increase in the available surface area at the air-water interface may allow more oil matter to adsorb at the air-water interface and be ejected into the atmosphere.²⁷

5.4.6 The effect of Corexit on the Rate of Ejection of Particulate IVOCs, SVOCs and NVOCs from NaCl Solution. The ejection of the particulate only fraction of alkanes (i.e., ESP collection) was greatest for IVOCs followed by SVOCs and NVOCs for Corexit 9527A and 9500A (Table S1). Across treatments, the ejection of the particulate only fraction of alkanes ranged from 0.07-0.67, 0.18-0.39, and 0.10-0.30 $\mu\text{g}\cdot\text{min}^{-1}$ for IVOCs, SVOCs, and NVOCs, respectively (Table S1).

The addition of Corexit 9527A resulted in a significant, at the 95 percent confidence interval, increase in the normalized rate of ejection of particulate (ESP collection) IVOCs, SVOCs, and NVOCs from NaCl solution relative to the oil only control (Table 2). The percent increase in the mean normalized rate of ejection of alkanes from NaCl solution in the presence of 9527A was 490%, 90%, and 160% for IVOCs, SVOCs, and NVOCs, respectively (Table 2). Similarly, the addition of Corexit 9500A also resulted in a significant increase in the normalized rates of ejection of particulate IVOCs, SVOCs, and NVOCs from NaCl solution relative to the oil only control (Table 2). The mean increase in the normalized rate of ejection in the presence of Corexit 9500A was 350%, 110% and 130% for IVOCs, SVOCs, and NVOCs, respectively (Table 2). The percent increase in the normalized rate of ejection of IVOCs measured by ESP was significantly, at the 95 percent confidence interval, higher than that of IVOCs measured by nozzle for both Corexit formulations (Table 2). This difference is due to the fact that in the nozzle collection the absolute rates of ejection of IVOCs are

dominated by the ejection of vapor phase alkanes. However, there was no significant difference in the normalized rate of ejection of SVOCs and NVOCs between collection methods (Table 2), indicating that SVOCs and NVOCs are ejected to the atmosphere as aerosols.

5.4.7 The Effect of Seawater on the Rates of Ejection of VOCs, IVOCs, SVOCs and NVOCs. Both Corexit formulations showed the same behavior with respect to the aerosolization of oil alkanes, therefore the aerosolization experiments were repeated in seawater using one Corexit formulation. Corexit 9500A was chosen because it was the dominant dispersant used during the DWH oil spill.⁴⁸ No significant difference in the normalized rate of ejection of VOCs from seawater was observed in the presence of Corexit 9500A relative to the oil only control (Table 2). This confirms the observations made for the ejection of VOCs from NaCl solution in the presence of Corexit 9500A.

The ejection of the combined volatile and particulate fraction of alkanes (nozzle collection) ranged from 8.1-11, and 0.47-1.1, 0.28-1.5 $\mu\text{g min}^{-1}$ for IVOCs, SVOCs, and NVOCs, respectively (Table S1). As was observed for NaCl solution, the presence of Corexit 9500A resulted in a significant increase in the normalized rates of ejection of the combined volatile and particulate fraction of IVOCs, SVOCs, and NVOCs from seawater relative to the oil only control (Table 2). The mean increase in the normalized rate of ejection of the combined volatile and particulate fraction of alkanes was 30%, 100% and 540%, for IVOCs, SVOCs and NVOCs, respectively (Table 2). There was no significant difference in the combined volatile and particulate normalized rates of ejection of IVOCs and SVOCs or NVOCs in the presence of Corexit 9500A between seawater and NaCl solution (Table 2).

The ejection of the particulate only fraction of alkanes (ESP collection) ranged from 0.10-0.34, 0.17-0.40 and 0.14-0.36 $\mu\text{g min}^{-1}$ for IVOCs, SVOCs, and NVOCs (Table S1). As was observed for NaCl solution, the ejection of the particulate only fraction of alkanes from seawater increased in the presence of Corexit 9500A (Table 2). The mean increase in the rate of ejection of the particulate only fraction of alkanes was 100%, 50%, and 70% for IVOCs, SVOCs and NVOCs, respectively (Table 2). Importantly, the same ejection behavior for all groups of alkane in seawater and NaCl

solution indicates that the latter can be used as a simple media to study the surface-driven transport of oil components to the atmosphere via bursting bubbles

5.5 Implications

The atmospheric transport of the surfactant components of Corexit used during the DWH oil spill via bursting bubbles was investigated and quantified in a laboratory scale aerosolization reactor. Oil premixed with Corexit 9527A or 9500A at a dispersant-to oil ratio (DOR) of 1:20, was injected at two different rates into a laboratory bubble column reactor filled with either NaCl solution or seawater. The ejection of Corexit surfactants associated with particulate matter via bursting bubbles was verified by the detection of DOSS, Tween 80 and Tween 85 in aerosol samples taken at the top of the column. The ratio of DOSS to Tween 80 and Tween 85 in the aerosol samples increased significantly (up to 50 fold) relative to their values in Corexit, which indicates the greater propensity for DOSS to be adsorbed at the air-water interface and ejected into the air upon bubble bursting relative to Tween 80 and Tween 85. Additionally, replacing the NaCl solution with seawater tends to increase the ejection rate of surfactants into the air.

In addition to the atmospheric transport of surfactants, the effect of Corexit 9527A and 9500A on the ejection rate of alkanes from oil was studied. The ejection rates of VOCs was either 14% lower (Corexit 9527A), or was found to be unaffected (Corexit 9500A), as compared to the rates measured when oil without dispersant was injected in the column. Both Corexit 9527A and 9500A significantly enhanced the ejection rates of IVOCs, SVOCs, and NVOCs relative to the oil-only control. No significant differences in the ejection rates of alkanes were observed between the two Corexit formulations, or when using NaCl solution or seawater.

This study highlights the importance of aerosolization phenomena when dispersants are used to mitigate oil spills. The detection of Corexit surfactants and alkane hydrocarbons in the aerosol phase has human health implications. Inhalation of particulate phase Corexit surfactants may affect emergency response workers and near shore residents during an emergency oil spill. Further study will be needed to assess the degree of human health risk associated with the inhalation of surfactant and hydrocarbon

aerosols during an emergency oil spill response, and to assess the potential for the long range transport of these aerosols.

5.6 Literature Cited

1. K. A. Kvenvolden; C. K. Cooper, Natural seepage of crude oil into the marine environment. *Geo-marine Letters* 2003, 23, (3-4), 140-146.
2. One Year Later Press Pack.
<http://www.restorethegulf.gov/release/2011/04/10/one-year-later-press-pack>
3. U.S. Chemical safety and hazard investigation board Explosion and fire at the Macondo well; 2014.
4. I. R. McDonald, Natural oil spills. *Scientific American* 1998, 279, 56-61.
5. I. R. MacDonald; N. L. Guinasso; S. G. Ackleson; J. F. Amos; R. Duckworth; R. Sassen; J. M. Brooks, Natural oil slicks in the Gulf off Mexico visible from space. *Journal of Geophysical Research* 1993, 98, (C9), 16,351–16,364.
6. Committee on Understanding Oil Spill Dispersants: Efficacy and effects; Council, N. R., *Understanding Oil Spill Dispersants: Efficacy and Effects*. The National Academies Press: 2005.
7. R. R. Lessard; DeMarco, G., The Significance of Oil Spill Dispersants. *Spill Science & Technology Bulletin* 2000, 6, (1), 59-68.
8. B. Lehr; S. Bristol; A. Possolo Oil Budget Calculator Deepwater Horizon.
http://www.restorethegulf.gov/sites/default/files/documents/pdf/OilBudgetCalc_Full_HQ-Print_111110.pdf
9. J. L. Gray; L. K. Kanagy; E. T. Furlong; C. J. Kanagy; J. W. McCoy; A. Mason; Lauenstein, G., Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. *Chemosphere* 2014, 95, (2014), 124-130.
10. J.L. Gray; L.K. Kanagy; E.T. Furlong; J.W. McCoy; C.J. Kanagy Determination of the Anionic Surfactant Di(Ethylhexyl) Sodium Sulfosuccinate in Water Samples Collected from Gulf of Mexico Coastal Waters Before and After Landfall of Oil from the Deepwater Horizon Oil Spill, May to October, 2010; U.S. Geological Survey 2011.

11. R. C. Prince; Butler, J. D., A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil. *Environmental Science and Pollution Research International* 2014, 21, (16), 9506–9510.
12. R. Almeda; C. Hyatt; Buskey, E. J., Toxicity of dispersant Corexit 9500A and crude oil to marine microzooplankton. *Ecotoxicology and Environmental Safety* 2014, 106, (2014), 76-85.
13. C. F. Wise ; J. T.F. Wise; S. S. Wise; W. D. Thompson; J. P. Wise Jr.; J. P. Wise Sr., Chemical dispersants used in the Gulf of Mexico oil crisis are cytotoxic and genotoxic to sperm whale skin cells. *Aquatic Toxicology* 2014, 152, (2014), 335-340.
14. G. Coelho; J. Clark; Aurand, D., Toxicity testing of dispersed oil requires adherence to standardized protocols to assess potential real world effects. *Environmental Pollution* 2013, 177, (2013), 185-188.
15. C. H. Peterson; S. S. Anderson; G. N. Cherr; R. F. Ambrose; S. Anghera; S. Bay; M. Blum; R. Condon; T. A. Dean; M. Graham; M. Guzy; S. Hampton; S. Joye; J. Lambrinos; B. Mate; D. Meffert; S. P. Powers; P. Somasundaran; R. B. Spies; C. M. Taylor; R. Tjeerdema; Adams, E. E., A Tale of Two Spills: Novel Science and Policy Implications of an Emerging New Oil Spill Model. *BioScience* 2012, 62, (5), 461-469.
16. E. B. Kujawinski; M. C. Kido Soule; D. L. Valentine; A. K. Boysen; K. Longnecker; Redmond, M. C., Fate of Dispersants Associated with the Deepwater Horizon Oil Spill. *Environmental Science & Technology* 2011, 45, (4), 1298-1306.
17. B. J. Place; M. J. Perkins; E. Sinclair; A. L. Barsamian; P. R. Blakemore; J. A. Field, Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. *Deep Sea Research Part II: Topical Studies in Oceanography* 2014.
18. H. K. White; S. L. Lyons; S. J. Harrison; D. M. Findley; Y. Liu; E. B. Kujawinski, Long-term persistence of dispersants following the Deepwater Horizon oil spill. *Environmental Science & Technology Letters* 2014, 1, (7), 295-299.

19. M. O. Perkins; J. A. Field, Analysis of Corexit in GOM Waters and Sediments. In Poster presented at the Gulf of Mexico Oil Spill and Ecosystem Science Conference, New Orleans, Louisiana, 2012.
20. M. O. Perkins; J. A. Field, DOSS in GOM sediments. In Platform presented at the Gulf of Mexico Oil Spill and Ecosystem Science Conference, Mobile, Alabama, 2014.
21. P. Campo; A. D. Venosa; M. T. Suidan, Biodegradability of Corexit 9500 and dispersed South Louisiana crude oil at 5 and 25 C. *Environmental science & technology* 2013, 47, (4), 1960-1967.
22. C. M. Glover; S. P. Mezyk; K. G. Linden; Rosario-Ortiz, F. L., Photochemical degradation of Corexit components in ocean water. *Chemosphere* 2014, 111, (2014), 596-602.
23. X. Zhao; Y. Gong; S. E. O'Reilly; Zhao, D., Effects of oil dispersant on solubilization, sorption and desorption of polycyclic aromatic hydrocarbons in sediment-seawater systems. *Marine Pollution Bulletin* 2015, 92, (1-2), 160-169.
24. J. Kemsley, Reckoning With Oil Spills. *Chemical & Engineering News* July 13, 2015, 2015, pp 8-12.
25. A. M. Middlebrook; D. M. Murphy; R. Ahmadov; E. L. Atlas; R. Bahreini; D. R. Blake; J. Brioude; J. A. de Gouw; F. C. Fehsenfeld; G. J. Frost; J. S. Holloway; D. A. Lack; J. M. Langridge; R. A. Lueb; S. A. McKeen; J. F. Meagher; S. Meinardi; J. A. Neuman; J. B. Nowak; D. D. Parrish; J. Peischl; A. E. Perring; I. B. Pollack; J. M. Roberts; T. B. Ryerson; J. P. Schwarz; J. R. Spackman; C. Warneke; Ravishankara, A. R., Air quality implications of the Deepwater Horizon oil spill. *PNAS* 2011, 109, (50), 20280-20285.
26. de Gouw, J. A.; Middlebrook, A. M.; Warneke, C.; Ahmadov, R.; Atlas, E. L.; Bahreini, R.; Blake, D. R.; Brock, C. A.; Brioude, J.; Fahey, D. W.; Fehsenfeld, F. C.; Holloway, J. S.; Henaff, M. L.; Lueb, R. A.; McKeen, S. A.; Meagher, J. F.; Murphy, D. M.; Paris, C.; D. D. Parrish; Perring, A. E.; Pollack, I. B.; Ravishankara, A. R.; A. L. Robinson; Ryerson, T. B.; Schwarz, J. P.; Spackman, J. R.; Srinivasan, A.; Watts, L. A.,

Organic Aerosol Formation Downwind from the Deepwater Horizon Oil Spill. *Science* 2011, 331, (6022), 1295-1299.

27. F. S. Ehrenhauser; P. Avij; X. Shu; V. Dugas; I. Woodson; T. Liyana-Arachchi; Z. Zhang; F. R. Hung; Valsaraj, K. T., Bubble bursting as an aerosol generation mechanism during an oil spill in the deep-sea environment : Laboratory experimental demonstration of the transport pathway. *Environ. Sci.: Processes Impacts* 2014, 16, (1), 65-73.

28. T. P. Liyana-Arachchi; Z. Zhang; F. S. Ehrenhauser; P. Avij; K. T. Valsaraj; Hung, F. R., Bubble bursting as an aerosol generation mechanism during an oil spill in the deep-sea environment: Molecular dynamics simulations of oil alkanes and dispersants in atmospheric air/salt water interfaces. *Environmental Science: Processes & Impacts* 2014, 16, (1).

29. G. M. Hidy; Brock, J. R., *Topics in current aerosol research*. Pergamon Press: 1971; Vol. 2, p 168.

30. M. C. Spillane; E. C. Monahan; P. A. Bowyer; D. M. Doyle; Stabeno, P. J., Whitecaps and global fluxes. In *Oceanic Whitecaps: And Their Role in Air-Sea Exchange Processes* Monahan, E. C.; Niocaill, G. M., Eds. D. Reidel Publishing Company: Dordrecht, Holland, 1986; pp 209-218.

31. D. C. Blanchard, The Production, Distribution, and Bacterial Enrichment of the Sea-Salt Aerosol. In *Air-Sea Exchange of Gases and Particles*, Liss, P. S.; Slinn, W. G. N., Eds. 1983; Vol. 108, pp 407-454.

32. R. Saint-Louis; Pelletier, E., Sea-to-air flux of contaminants via bubbles bursting. An experimental approach for tributyltin. *Marine Chemistry* 2003, 84, (2004), 211-224.

33. Ramirez, C. E.; Batchu, S. R.; Gardinali, P. R., High sensitivity liquid chromatography tandem mass spectrometric methods for the analysis of dioctyl sulfosuccinate in different stages of an oil spill response monitoring effort. *Analytical and Bioanalytical Chemistry* 2013, 405, (12), 4167-4175.

34. B. J. Place; M. J. Perkins; E. Sinclair; A. L. Barsamian; P. R. Blakemore; Field, J. A., Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. Deep-Sea Research 2014.
35. M. Fontana, An Aspect of Coastal Pollution- The Combined Effect of Detergent and Oil at Sea on Sea Spray Composition. Water, Air & Soil Pollution 1976, 5, (3), 269-280.
36. C. J. Mcmurdo; D. A. Ellis; E. Webster; J. Butler; R. D. Christensen; Reid, L. K., Aerosol Enrichment of the Surfactant PFO and Mediation of the Water-Air Transport of Gaseous PFOA. Environmental Science & Technology 2008, 42, (11), 3969–3974.
37. H. Tervahattu; K. Hartonen; V. Kerminen; K. Kupiainen; P. Aarnio; T. Koskentalo; A. F. Tuck; Vaida, V., New evidence of an organic layer on marine aerosols. JOURNAL OF GEOPHYSICAL RESEARCH 2002, 107, (D7).
38. C. Oppo; S. Bellandi; N. Degli Innocenti; A.M. Stortini; G. Loglio; E. Schiavuta; Cini, R., Surfactant components of marine organic matter as agents for biogeochemical fractionation and pollutant transport via marine aerosols. Marine Chemistry 1998, 63, (3-4), 235–253.
39. J. S. Smith; L. F. Burns; K. T. Valsaraj; Thibodeaux, L. J., Bubble Column Reactors for Wastewater Treatment. 2. The Effect of Sparger Design on Sublation Column Hydrodynamics in the Homogeneous Flow Regime. Industrial and Engineering Chemistry Research 1996, 35, (5), 1700-1710.
40. W. C. Keene; H. Maring; J. R. Maben; D. J. Kieber; A. A. P. Pszenny; E. E. Dahl; M. A. Izaguirre; A. J. Davis; M. S. Long; X. Zhou; L. Smoydzin; Sander, R., Chemical and physical characteristics of nascent aerosols produced by bursting bubbles at a model air-sea interface. Journal of Geophysical Research 2007, 112, (D21).
41. F. J. Millero; R. Feistel; D. G. Wright; T. J. McDougall, The composition of Standard Seawater and the definition of the Reference-Composition Salinity Scale. Deep-Sea Research 2008, 50–72.

42. THE MERCK INDEX Eleventh Edition. In Chemical & Engineering News Archive, American Chemical Society: 1989; Vol. 67.
43. Gray, J. L.; Kanagy, L. K.; Furlong, E. T.; Kanagy, C. J.; McCoy, J. W.; Mason, A.; Lauenstein, G., Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. 2014, 95, 124-130.
44. L. Theodore, Air Pollution Control Equipment Calculations. Wiley: 2008; p 574.
45. S. J. Pogorzelski; A. D. Kogut; A. Z. Mazurek, Surface rheology parameters of source-specific surfactant films as indicators of organic matter dynamics. *Hydrobiologia* 2006, 554, (2006), 67-81.
46. M. A. McInnes; D. A. Ellis; E. M. Webster; A. Peters, Sea spray and the atmospheric transport of nonylphenol ethoxylates. A combined laboratory, field and modeling study. *Atmospheric Environment* 2013, 69, 304-312.
47. Council, N. R., Oil Spill Dispersants: Efficacy and Effects. The National Academies Press: Washington, DC, 2005; p 400.
48. (OSAT), O. S. A. T., Summary Report for SubSea and SubSurface Oil and Dispersant Detection: Sampling and Monitoring. DIANE Publishing: 2010.

CHAPTER 6 – PARTING WORDS

6.1 The Big Picture.

In response to the DWH oil spill the first deep-sea application of chemical oil dispersant as well as the application of unprecedented quantities of chemical dispersant to surface-oil slicks occurred.¹ The dispersant of choice, Corexit, is a complex mixture with individual components that have unique polarity and solubility.² At the time of application, significant data gaps existed as to the likely transport, persistence and ecosystem impact that would result from the individual components of Corexit.³ In the aftermath of DWH, the design of laboratory experiments largely focused on crude oil and did not adequately address Corexit as a complex mixture. For example, many experiments used Corexit-only and CEWAF mixtures in laboratory exposures designed to disentangle the toxicity of Corexit from that of crude oil in CEWAF.⁴⁻¹⁰ These experiments often treated Corexit-only solutions as controls for CEWAF but did not quantify the individual components of Corexit in CEWAF.

These studies may have relied on bulk dilution calculations or the quantification of a single component of Corexit because the analytical methodology for the quantification of the individual components of Corexit had to be developed in response to the needs for environmental monitoring following DWH.¹¹ The development of analytical methods focused on seawater, leaving a need for the development of quantitative methods for marine sediment.^{2, 12-19} The work presented in this thesis addressed the complexity of experimental design with a focus on the individual surfactant components of Corexit. Specifically, differences in the initial surfactant composition between Corexit-only and CEWAF mixtures were observed to result from an experimental design widely used in the scientific community to generate CEWAF. Additionally, remaining methodological needs were addressed with the development of a method for the quantification of DOSS in marine

sediments. This method was applied to sediments in the Gulf to define the spatial footprint of DOSS impacted sediments and temporal trends in DOSS concentration from 2010 through 2015.

6.2 Method Innovation, Application and Future Work.

The laboratory microcosm study described in Chapter 2 quantified the extent of primary microbial degradation of the Corexit surfactants in laboratory microcosms using seawater collected at depth (1,178 m) near a natural hydrocarbon seep in the Gulf. Primary degradation of the non-ionics was rapid (<7 d). However, primary degradation of DOSS was significant but incomplete (33-41%) over the course of incubations. Mass balance was not achieved between DOSS and the transformation intermediate EHSS, indicating that other transformation products were present. Identification and quantification of these intermediates was beyond the scope of the work presented in this thesis. Seidel et al. have proposed possible additional intermediates of DOSS degradation but did not quantify these compounds.²⁰ Future work could focus on the identification and quantification of other important transformation intermediates of DOSS. Additional work should also characterize the presence or absence of proposed transformation intermediates in the off-the-shelf Corexit formulation. For example, EHSS is a microbial and abiotic hydrolysis product of DOSS and is present in the whole Corexit.^{2, 20}

Chapter 2 also describes significant differences in the ratio of the individual Corexit surfactants between time zero Corexit-only and CEWAF treatments which indicates that the Corexit surfactants have individual tendencies to associate with free-phase oil or seawater during CEWAF formation. Surfactant associated with free-phase-oil is then excluded from the resulting aqueous solution when the oil is discarded. Chapter 2 also describes additional experiments undertaken to quantify the abiotic hydrolysis of DOSS at 25° C. No significant abiotic losses were observed over 28 days. This observation is in contrast to that reported by Campo et al. and suggests that the abiotic loss of DOSS observed by Campo et al. was due to the addition of sodium azide as a biocide. Alternatively, future work could aim to identify parameters

that control the availability of freely dissolved surfactant in a CEWAF. For example, does the concentration of dissolved organic carbon effect the concentration of free surfactant?

Chapters 3 and 4 describe a method for the quantification for DOSS in marine solids by LC-QTOF, the application of the method to sediments and sediment trap solids collected in the Gulf, and the spatial and temporal trends in DOSS concentration in deep-sea Gulf sediments. Chapter 3 discusses interferences observed for DOSS and its isotopes that compromise the quantification of DOSS in some samples when analyzed by LC-MS/MS. These interferences were resolved without further sample clean up by taking advantage of the greater selectivity of QTOF. Chapter 3 also presents method performance metrics which were previously missing from the literature. Chapter 4 presents the application of that method to a larger number of sediments collected between 2010 and 2015 and incorporated publicly-available NRDA data to define the spatial foot print of DOSS-impacted gulf sediments and temporal trends in sediment DOSS concentrations. Chapter 4 presents the largest spatial dataset in the literature. Additionally, DOSS-impacted sediments were observed in 2015, documenting the long term persistence of DOSS in sediments within 13 km from DWH more than 5 years after the DWH application of Corexit. Future work could include analysis of a greater number of samples to better define the temporal trends of DOSS concentration. Alternatively, future work could target the analysis of archived samples for the non-ionic surfactants, which in the absence of transformation, may be expected to associate with surface oil due to greater hydrophobicity.

Chapter 5 describes the ejection of surfactant aerosols from Corexit-oil mixtures under conditions that occur during breaking waves. This has not been previously identified as a transport pathway for the Corexit surfactants and has implications for the potential exposure of emergency responders to Corexit aerosols. Additionally, this work confirms that the presence of Corexit at a relevant dispersant to oil ratio increases the rate of ejection of semi and nonvolatile alkanes. Future research could focus on risk assessment for emergency responder exposure.

Oil spills will continue to occur and the use of chemical dispersant will likely remain a key emergency oil spill response tool. Corexit 9500 will likely be the dispersant of choice due to its availability and its large volume use during the DWH emergency response. Therefore, it is essential that the transport, persistence, and impact of Corexit in the marine environment be fully documented. This research solved critical analytical challenges in surfactant measurement and informed the transport and persistence of surfactants in marine systems.

6.3 Literature Cited.

1. Operational Science Advisory Team Unified Area Command. Summary Report for Sub-Sea and Sub-Surface Oil and Dispersant Detection: Sampling and Monitoring. **2010.**
2. Place, B. J.; Perkins, M. J.; Sinclair, E.; Barsamian, A. L.; Blakemore, P. R.; Field, J. A., Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. *Deep Sea Research Part II: Topical Studies in Oceanography* **2014.**
3. Deepwater Horizon oil spill: Final Programmatic Damage Assessment and Restoration Plan and Final Programmatic Environmental Impact Statement. Retrieved from <http://www.gulfspillrestoration.noaa.gov/restoration-planning/gulf-plan> **Deepwater Horizon Natural Resource Damage Assessment Trustees (2016).**
4. Cohen, J. H.; McCormick, L. R.; Burkhardt, S. M., Effects of dispersant and oil on survival and swimming activity in a marine copepod. *Bulletin of environmental contamination and toxicology* **2014**, 92, (4), 381-387.
5. Forth, H. P.; Mitchelmore, C. L.; Morris, J. M.; Lipton, J., Characterization of oil and water accommodated fractions used to conduct aquatic toxicity testing in support of the Deepwater Horizon oil spill natural resource damage assessment. *Environmental Toxicology and Chemistry* **2015.**
6. Singer, M. M.; George, S.; Jacobson, S.; Lee, I.; Weetman, L. L.; Tjeerdema, R. S.; Sowby, M. L., Comparison of acute aquatic effects of the oil dispersant Corexit 9500 with those of other Corexit series dispersants. *Ecotoxicology and Environmental Safety* **1996**, 35, (2), 183-189.
7. Özhan, K.; Miles, S. M.; Gao, H.; Bargu, S., Relative Phytoplankton growth responses to physically and chemically dispersed South Louisiana sweet crude oil. *Environmental monitoring and assessment* **2014**, 186, (6), 3941-3956.
8. Anderson, J. A.; Kuhl, A. J.; Anderson, A. N., Toxicity of oil and dispersed oil on juvenile mud crabs, *Rhithropanopeus harrisi*. *Bulletin of environmental contamination and toxicology* **2014**, 92, (4), 375-380.
9. Bejarano, A. C.; Clark, J. R.; Coelho, G. M., Issues and challenges with oil toxicity data and implications for their use in decision making: A quantitative review. *Environmental toxicology and chemistry* **2014**, 33, (4), 732-742.
10. Adams, J.; Sweezey, M.; Hodson, P. V., Oil and oil dispersant do not cause synergistic toxicity to fish embryos. *Environmental toxicology and chemistry* **2014**, 33, (1), 107-114.
11. Place, B.; Anderson, B.; Mekebri, A.; Furlong, E. T.; Gray, J. L.; Tjeerdema, R.; Field, J., A role for analytical chemistry in advancing our understanding of the occurrence, fate, and effects of Corexit oil dispersants. In ACS Publications: 2010.

12. Kujawinski, E. B.; Soule, M. C. K.; Valentine, D. L.; Boysen, A. K.; Longnecker, K.; Redmond, M. C., Fate of Dispersants Associated with the Deepwater Horizon Oil Spill. *Environ Sci Technol* **2011**, *45*, (4), 1298-1306.
13. Hayworth, J. S.; Clement, T. P., Provenance of Corexit-related chemical constituents found in nearshore and inland Gulf Coast waters. *Marine pollution bulletin* **2012**, *64*, (10), 2005-2014.
14. Mathew, J.; Schroeder, D. L.; Zintek, L. B.; Schupp, C. R.; Kosempa, M. G.; Zachary, A. M.; Schupp, G. C.; Wesolowski, D. J., Dioctyl sulfosuccinate analysis in near-shore Gulf of Mexico water by direct-injection liquid chromatography-tandem mass spectrometry. *J Chromatogr A* **2012**, *1231*, 46-51.
15. Ramirez, C. E.; Batchu, S. R.; Gardinali, P. R., High sensitivity liquid chromatography tandem mass spectrometric methods for the analysis of dioctyl sulfosuccinate in different stages of an oil spill response monitoring effort. *Anal Bioanal Chem* **2013**, *405*, (12), 4167-4175.
16. King, A.; Readman, J.; Zhou, J., Determination of polycyclic aromatic hydrocarbons in water by solid-phase microextraction–gas chromatography–mass spectrometry. *Analytica chimica acta* **2004**, *523*, (2), 259-267.
17. Gray, J. L.; Kanagy, L. K.; Furlong, E. T.; Kanagy, C. J.; McCoy, J. W.; Mason, A.; Lauenstein, G., Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. **2014**, *95*, 124-130.
18. Brunswick, P.; Shang, D.; van Aggelen, G.; Buday, C.; Kim, M., Rapid and Sensitive LC/MS/MS Direct Injection Method for the Determination of Trace Level Corexit EC9500A Oil Dispersant in Seawater. *Environmental Forensics* **2015**, *16*, (4), 333-343.
19. Yeudakimau, A. V.; Perkins, C. R.; Guerrero, G. M.; Stuart, J. D.; Provatas, A. A., QuEChERS sample preparation followed by ultra-performance liquid chromatography–tandem mass spectrometry for rapid screening of dioctyl sulfosuccinate sodium salt in avian egg tissue. *International Journal of Environmental Analytical Chemistry* **2014**, *94*, (12), 1183-1198.
20. Seidel, M.; Kleindienst, S.; Dittmar, T.; Joye, S. B.; Medeiros, P. M., Biodegradation of crude oil and dispersants in deep seawater from the Gulf of Mexico: Insights from ultra-high resolution mass spectrometry. *Deep Sea Research Part II: Topical Studies in Oceanography* **2016**, *129*, 108-118.

BIBLIOGRAPHY

National Research Council. Dispersant Oil Spill Efficacy and Effects. **2005**.

National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling. The Use of Surface and Subsea Dispersants During the BP Deepwater Horizon Oil Spill. **2011**.

Deepwater Horizon oil spill: Final Programmatic Damage Assessment and Restoration Plan and Final Programmatic Environmental Impact Statement. Retrieved from <http://www.gulfspillrestoration.noaa.gov/restoration-planning/gulf-plan> Deepwater Horizon Natural Resource Damage Assessment Trustees. **2016**.

Operational Science Advisory Team Unified Area Command. Summary Report for Sub-Sea and Sub-Surface Oil and Dispersant Detection: Sampling and Monitoring. **2010**.

Adams, J.; Sweezey, M.; Hodson, P. V., Oil and oil dispersant do not cause synergistic toxicity to fish embryos. *Environmental toxicology and chemistry* **2014**, 33, (1), 107-114.

Almeda, R.; Hyatt, C.; Buskey, E. J., Toxicity of dispersant Corexit 9500A and crude oil to marine microzooplankton. *Ecotoxicology and environmental safety* **2014**, 106, 76-85.

Anderson, J. A.; Kuhl, A. J.; Anderson, A. N., Toxicity of oil and dispersed oil on juvenile mud crabs, *Rhithropanopeus harrisi*. *Bulletin of environmental contamination and toxicology* **2014**, 92, (4), 375-380.

Annesley, T. M., Ion suppression in mass spectrometry. *Clinical chemistry* **2003**, 49, (7), 1041-1044.

Bælum, J.; Borglin, S.; Chakraborty, R.; Fortney, J. L.; Lamendella, R.; Mason, O. U.; Auer, M.; Zemla, M.; Bill, M.; Conrad, M. E., Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. *Environmental microbiology* **2012**, 14, (9), 2405-2416.

Baguley, J. G.; Montagna, P. A.; Cooksey, C.; Hyland, J. L.; Bang, H. W.; Morrison, C.; Kamikawa, A.; Bennetts, P.; Saiyo, G.; Parsons, E., Community response of deep-sea soft-sediment metazoan meiofauna to the Deepwater Horizon blowout and oil spill. *Marine Ecology Progress Series* **2015**, 528, 127-140.

Barsamian, A. L.; Perkins, M. J.; Field, J. A.; Blakemore, P. R., Regioselective syntheses of [^{13}C] 4-labelled sodium 1-carboxy-2-(2-ethylhexyloxycarbonyl) ethanesulfonate and sodium 2-carboxy-1-(2-ethylhexyloxycarbonyl) ethanesulfonate from [^{13}C] 4-maleic anhydride. *Journal of Labelled Compounds and Radiopharmaceuticals* **2014**, 57, (5), 397-401.

Batchu, S. R.; Ramirez, C. E.; Gardinali, P. R., Stability of dioctyl sulfosuccinate (DOSS) towards hydrolysis and photodegradation under simulated solar conditions. *Science of the Total Environment* **2014**, 481, 260-265.

Bejarano, A. C.; Clark, J. R.; Coelho, G. M., Issues and challenges with oil toxicity data and implications for their use in decision making: A quantitative review. *Environmental toxicology and chemistry* **2014**, 33, (4), 732-742.

Beyer, J.; Trannum, H. C.; Bakke, T.; Hodson, P. V.; Collier, T. K., Environmental effects of the Deepwater Horizon oil spill: a review. *Marine Pollution Bulletin* **2016**.

Borisov, O. V.; Ji, J. A.; Wang, Y. J.; Vega, F.; Ling, V. T., Toward understanding molecular heterogeneity of polysorbates by application of liquid chromatography–mass spectrometry with computer-aided data analysis. *Analytical chemistry* **2011**, 83, (10), 3934-3942.

Brunswick, P.; Shang, D.; van Aggelen, G.; Buday, C.; Kim, M., Rapid and Sensitive LC/MS/MS Direct Injection Method for the Determination of Trace Level Corexit EC9500A Oil Dispersant in Seawater. *Environmental Forensics* **2015**, 16, (4), 333-343.

Burford, M. D.; Hawthorne, S. B.; Miller, D. J., Extraction rates of spiked versus native PAHs from heterogeneous environmental samples using supercritical fluid extraction and sonication in methylene chloride. *Analytical Chemistry* **1993**, 65, (11), 1497-1505.

Camilli, R.; Reddy, C. M.; Yoerger, D. R.; Van Mooy, B. A.; Jakuba, M. V.; Kinsey, J. C.; McIntyre, C. P.; Sylva, S. P.; Maloney, J. V., Tracking hydrocarbon plume transport and biodegradation at Deepwater Horizon. *Science* **2010**, 330, (6001), 201-204.

Campo, P.; Venosa, A. D.; Suidan, M. T., Biodegradability of Corexit 9500 and dispersed South Louisiana crude oil at 5 and 25 C. *Environ Sci Technol* **2013**, 47, (4), 1960-1967.

Chanton, J.; Zhao, T.; Rosenheim, B. E.; Joye, S.; Bosman, S.; Brunner, C.; Yeager,

K. M.; Diercks, A. R.; Hollander, D., Using natural abundance radiocarbon to trace the flux of petrocarbon to the seafloor following the Deepwater Horizon oil spill. *Environ Sci Technol* **2014**, 49, (2), 847-854.

Cohen, J. H.; McCormick, L. R.; Burkhardt, S. M., Effects of dispersant and oil on survival and swimming activity in a marine copepod. *Bulletin of environmental contamination and toxicology* **2014**, 92, (4), 381-387.

Crescenzi, C.; Di Corcia, A.; Samperi, R.; Marcomini, A., Determination of nonionic polyethoxylate surfactants in environmental waters by liquid chromatography/electrospray mass spectrometry. *Analytical Chemistry* **1995**, 67, (11), 1797-1804.

Daly, K. L.; Passow, U.; Chanton, J.; Hollander, D., Assessing the impacts of oil-associated marine snow formation and sedimentation during and after the Deepwater Horizon oil spill. *Anthropocene* **2016**.

DeLeo, D. M.; Ruiz-Ramos, D. V.; Baums, I. B.; Cordes, E. E., Response of deep-water corals to oil and chemical dispersant exposure. *Deep Sea Research Part II: Topical Studies in Oceanography* **2016**, 129, 137-147.

Ehrenhauser, F. S.; Avij, P.; Shu, X.; Dugas, V.; Woodson, I.; Liyana-Arachchi, T.; Zhang, Z.; Hung, F. R.; Valsaraj, K. T., Bubble bursting as an aerosol generation mechanism during an oil spill in the deep-sea environment: laboratory experimental demonstration of the transport pathway. *Environmental Science: Processes & Impacts* **2014**, 16, (1), 65-73.

Erdem, N. S.; Alawani, N.; Wesdemiotis, C., Characterization of polysorbate 85, a nonionic surfactant, by liquid chromatography vs. ion mobility separation coupled with tandem mass spectrometry. *Analytica chimica acta* **2014**, 808, 83-93.

Fisher, C. R.; Hsing, P.-Y.; Kaiser, C. L.; Yoerger, D. R.; Roberts, H. H.; Shedd, W. W.; Cordes, E. E.; Shank, T. M.; Berlet, S. P.; Saunders, M. G., Footprint of Deepwater Horizon blowout impact to deep-water coral communities. *Proceedings of the National Academy of Sciences* **2014**, 111, (32), 11744-11749.

Forth, H. P.; Mitchelmore, C. L.; Morris, J. M.; Lipton, J., Characterization of oil and water accommodated fractions used to conduct aquatic toxicity testing in support of the Deepwater Horizon oil spill natural resource damage assessment. *Environmental Toxicology and Chemistry* **2015**.

Garcia, M.; Campos, E.; Marsal, A.; Ribosa, I., Biodegradability and toxicity of

sulphonate-based surfactants in aerobic and anaerobic aquatic environments. *Water Research* **2009**, *43*, (2), 295-302.

George-Ares, A.; Clark, J., Aquatic toxicity of two Corexit® dispersants. *Chemosphere* **2000**, *40*, (8), 897-906.

Glover, C. M.; Mezyk, S. P.; Linden, K. G.; Rosario-Ortiz, F. L., Photochemical degradation of Corexit components in ocean water. *Chemosphere* **2014**, *111*, 596-602.

Gosetti, F.; Mazzucco, E.; Zampieri, D.; Gennaro, M. C., Signal suppression/enhancement in high-performance liquid chromatography tandem mass spectrometry. *J Chromatogr A* **2010**, *1217*, (25), 3929-3937.

Gray, J. L.; Kanagy, L. K.; Furlong, E. T.; Kanagy, C. J.; McCoy, J. W.; Mason, A.; Lauenstein, G., Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. **2014**, *95*, 124-130.

Hales, S. G., Biodegradation of the anionic surfactant dialkyl sulphosuccinate. *Environmental toxicology and chemistry* **1993**, *12*, (10), 1821-1828.

Hatzinger, P. B.; Alexander, M., Effect of aging of chemicals in soil on their biodegradability and extractability. *Environ Sci Technol* **1995**, *29*, (2), 537-545.

Hayworth, J. S.; Clement, T. P., Provenance of Corexit-related chemical constituents found in nearshore and inland Gulf Coast waters. *Marine pollution bulletin* **2012**, *64*, (10), 2005-2014.

Jewett, S. C.; Dean, T. A.; Smith, R. O.; Blanchard, A., 'Exxon Valdez' oil spill: impacts and recovery in the soft-bottom benthic community in and adjacent to eelgrass beds. *Marine Ecology Progress Series* **1999**, *185*, 59-83.

Joye, S. B.; Bowles, M. W.; Samarkin, V. A.; Hunter, K. S.; Niemann, H., Biogeochemical signatures and microbial activity of different cold-seep habitats along the Gulf of Mexico deep slope. *Deep Sea Research Part II: Topical Studies in Oceanography* **2010**, *57*, (21), 1990-2001.

Kaufmann, A.; Butcher, P.; Maden, K.; Widmer, M.; Giles, K.; Uría, D., Are liquid chromatography/electrospray tandem quadrupole fragmentation ratios unequivocal confirmation criteria? *Rapid Communications in Mass Spectrometry* **2009**, *23*, (7), 985-998.

Kleindienst, S.; Seidel, M.; Ziervogel, K.; Grim, S.; Loftis, K.; Harrison, S.; Malkin, S. Y.; Perkins, M. J.; Field, J.; Sogin, M. L., Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. *Proceedings of the National Academy of Sciences* **2015**, 201507380.

Kover, S. C.; Rosario-Ortiz, F. L.; Linden, K. G., Photochemical fate of solvent constituents of Corexit oil dispersants. *Water research* **2014**, 52, 101-111.

Kujawinski, E. B.; Soule, M. C. K.; Valentine, D. L.; Boysen, A. K.; Longnecker, K.; Redmond, M. C., Fate of Dispersants Associated with the Deepwater Horizon Oil Spill. *Environ Sci Technol* **2011**, 45, (4), 1298-1306.

108. Lessard, R. R.; DeMarco, G., The significance of oil spill dispersants. *Spill Science & Technology Bulletin* **2000**, 6, (1), 59-68.

Mathew, J.; Schroeder, D. L.; Zintek, L. B.; Schupp, C. R.; Kosempa, M. G.; Zachary, A. M.; Schupp, G. C.; Wesolowski, D. J., Dioctyl sulfosuccinate analysis in near-shore Gulf of Mexico water by direct-injection liquid chromatography-tandem mass spectrometry. *J Chromatogr A* **2012**, 1231, 46-51.

Mitchell, F. M.; Holdway, D. A., The acute and chronic toxicity of the dispersants Corexit 9527 and 9500, water accommodated fraction (WAF) of crude oil, and dispersant enhanced WAF (DEWAF) to *Hydra viridissima* (green hydra). *Water Research* **2000**, 34, (1), 343-348.

Monahan, E. C.; Mac Niocaill, G., *Oceanic Whitecaps: And Their Role in Air-Sea Exchange Processes*. Springer Science & Business Media: 2012; Vol. 2.

Montagna, P. A.; Baguley, J. G.; Cooksey, C.; Hartwell, I.; Hyde, L. J.; Hyland, J. L.; Kalke, R. D.; Kracker, L. M.; Reuscher, M.; Rhodes, A. C., Deep-sea benthic footprint of the Deepwater Horizon blowout. *Plos One* **2013**, 8, (8), e70540.

Özhan, K.; Miles, S. M.; Gao, H.; Bargu, S., Relative Phytoplankton growth responses to physically and chemically dispersed South Louisiana sweet crude oil. *Environmental monitoring and assessment* **2014**, 186, (6), 3941-3956.

Paine, R. T.; Ruesink, J. L.; Sun, A.; Soulanille, E. L.; Wonham, M. J.; Harley, C. D.; Brumbaugh, D. R.; Secord, D. L., Trouble on oiled waters: lessons from the Exxon Valdez oil spill. *Annual Review of Ecology and Systematics* **1996**, 27, (1), 197-235.

Parker, A. M.; Ferrer, I.; Thurman, E. M.; Rosario-Ortiz, F. L.; Linden, K. G., Determination of COREXIT components used in the Deepwater Horizon cleanup by

liquid chromatography-ion trap mass spectrometry. *Anal Methods-Uk* **2014**, 6, (15), 5498-5502.

Passow, U., Formation of rapidly-sinking, oil-associated marine snow. *Deep Sea Research Part II: Topical Studies in Oceanography* **2014**.

Passow, U.; Ziervogel, K.; Asper, V.; Diercks, A., Marine snow formation in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. *Environmental Research Letters* **2012**, 7, (3), 035301.

Perkins, M. J.; Joye, S. B.; Field, J. A., Selective quantification of DOSS in marine sediment and sediment-trap solids by LC-QTOF-MS. *Anal Bioanal Chem* **2016**, 1-8.

Peterson, C. H.; Anderson, S. S.; Cherr, G. N.; Ambrose, R. F.; Anghera, S.; Bay, S.; Blum, M.; Condon, R.; Dean, T. A.; Graham, M., A tale of two spills: novel science and policy implications of an emerging new oil spill model. *BioScience* **2012**, 62, (5), 461-469.

Place, B.; Anderson, B.; Mekebri, A.; Furlong, E. T.; Gray, J. L.; Tjeerdema, R.; Field, J., A role for analytical chemistry in advancing our understanding of the occurrence, fate, and effects of Corexit oil dispersants. *Environ Sci Technol* **2010**, 44, (16), 6016-6018.

Place, B. J.; Perkins, M. J.; Sinclair, E.; Barsamian, A. L.; Blakemore, P. R.; Field, J. A., Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. *Deep Sea Research Part II: Topical Studies in Oceanography* **2014**.

Prince, R. C., Oil Spill Dispersants: Boon or Bane? *Environ Sci Technol* **2015**, 49, (11), 6376-6384.

Ramirez, C. E.; Batchu, S. R.; Gardinali, P. R., High sensitivity liquid chromatography tandem mass spectrometric methods for the analysis of dioctyl sulfosuccinate in different stages of an oil spill response monitoring effort. *Anal Bioanal Chem* **2013**, 405, (12), 4167-4175.

Reddy, C. M.; Arey, J. S.; Seewald, J. S.; Sylva, S. P.; Lemkau, K. L.; Nelson, R. K.; Carmichael, C. A.; McIntyre, C. P.; Fenwick, J.; Ventura, G. T., Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. *Proceedings of the National Academy of Sciences* **2012**, 109, (50), 20229-20234.

Rico-Martínez, R.; Snell, T. W.; Shearer, T. L., Synergistic toxicity of Macondo

crude oil and dispersant Corexit 9500A® to the *Brachionus plicatilis* species complex (Rotifera). *Environmental Pollution* **2013**, 173, 5-10.

Riehm, D. A.; McCormick, A. V., The role of dispersants' dynamic interfacial tension in effective crude oil spill dispersion. *Marine pollution bulletin* **2014**, 84, (1), 155-163.

Romero, I. C.; Schwing, P. T.; Brooks, G. R.; Larson, R. A.; Hastings, D. W.; Ellis, G.; Goddard, E. A.; Hollander, D. J., Hydrocarbons in deep-sea sediments following the 2010 Deepwater Horizon blowout in the northeast Gulf of Mexico. *Plos One* **2015**, 10, (5), e0128371.

Sancho, J. V.; Pozo, Ó. J.; Ibáñez, M.; Hernández, F., Potential of liquid chromatography/time-of-flight mass spectrometry for the determination of pesticides and transformation products in water. *Anal Bioanal Chem* **2006**, 386, (4), 987-997.

Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J., Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ Sci Technol* **2014**, 48, (4), 2097-2098.

Seidel, M.; Kleindienst, S.; Dittmar, T.; Joye, S. B.; Medeiros, P. M., Biodegradation of crude oil and dispersants in deep seawater from the Gulf of Mexico: Insights from ultra-high resolution mass spectrometry. *Deep Sea Research Part II: Topical Studies in Oceanography* **2015**.

Singer, M.; Aurand, D.; Bragin, G.; Clark, J.; Coelho, G.; Sowby, M.; Tjeerdema, R., Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. *Marine Pollution Bulletin* **2000**, 40, (11), 1007-1016.

Singer, M. M.; George, S.; Jacobson, S.; Lee, I.; Weetman, L. L.; Tjeerdema, R. S.; Sowby, M. L., Comparison of acute aquatic effects of the oil dispersant Corexit 9500 with those of other Corexit series dispersants. *Ecotoxicology and Environmental Safety* **1996**, 35, (2), 183-189.

Stefansson, E. S.; Langdon, C. J.; Pargee, S. M.; Blunt, S. M.; Gage, S. J.; Stubblefield, W. A., Acute effects of non-weathered and weathered crude oil and dispersant associated with the deepwater horizon incident on the development of marine bivalve and echinoderm larvae. *Environmental Toxicology and Chemistry* **2016**.

Stout, S. A.; Payne, J. R., Macondo oil in deep-sea sediments: Part 1—sub-sea weathering of oil deposited on the seafloor. *Marine Pollution Bulletin* **2016**.

Stout, S. A.; Rouhani, S.; Liu, B.; Oehrig, J.; Ricker, R. W.; Baker, G.; Lewis, C., Assessing the footprint and volume of oil deposited in deep-sea sediments following the Deepwater Horizon oil spill. *Marine Pollution Bulletin* **2016**.

Trufelli, H.; Palma, P.; Famiglini, G.; Cappiello, A., An overview of matrix effects in liquid chromatography–mass spectrometry. *Mass spectrometry reviews* **2011**, *30*, (3), 491-509.

Valentine, D. L.; Fisher, G. B.; Bagby, S. C.; Nelson, R. K.; Reddy, C. M.; Sylva, S. P.; Woo, M. A., Fallout plume of submerged oil from Deepwater Horizon. *Proceedings of the National Academy of Sciences* **2014**, *111*, (45), 15906-15911.

Vonk, S. M.; Hollander, D. J.; Murk, A. J., Was the extreme and wide-spread marine oil-snow sedimentation and flocculent accumulation (MOSSFA) event during the Deepwater Horizon blow-out unique? *Marine Pollution Bulletin* **2015**, *100*, (1), 5-12.

White, H. K.; Hsing, P.-Y.; Cho, W.; Shank, T. M.; Cordes, E. E.; Quattrini, A. M.; Nelson, R. K.; Camilli, R.; Demopoulos, A. W.; German, C. R., Impact of the Deepwater Horizon oil spill on a deep-water coral community in the Gulf of Mexico. *Proceedings of the National Academy of Sciences* **2012**, *109*, (50), 20303-20308.

White, H. K.; Lyons, S. L.; Harrison, S. J.; Findley, D. M.; Liu, Y.; Kujawinski, E. B., Long-Term Persistence of Dispersants following the Deepwater Horizon Oil Spill. *Environ Sci Technol Lett* **2014**, *1*, (7), 295-299.

Yan, B., Passow, U, Chanton, J3, Nöthig, EM Asper, V, Sweet, J. Pitiranggon, M, Diercks, A, Pak, D., Sustained deposition of contaminants from the Deepwater Horizon spill. *PNAS* **2015**, *In Press*.

Yeudakimau, A. V.; Perkins, C. R.; Guerrero, G. M.; Stuart, J. D.; Provatas, A. A., QuEChERS sample preparation followed by ultra-performance liquid chromatography–tandem mass spectrometry for rapid screening of dioctyl sulfosuccinate sodium salt in avian egg tissue. *International Journal of Environmental Analytical Chemistry* **2014**, *94*, (12), 1183-1198.

Ziervogel, K.; Joye, S. B.; Arnosti, C., Microbial enzymatic activity and secondary production in sediments affected by the sedimentation pulse following the Deepwater Horizon oil spill. *Deep Sea Research Part II: Topical Studies in Oceanography* **2014**.

APPENDICIES

**Appendix 1 – Chapter 2 Supporting Information: Corexit Surfactant
Biodegradation, Hydrolysis and Aqueous Phase Composition After CEWAF
Formation**

Table A1.1. DOSS, EHSS, Span 80 and Tween 80 and Tween 85 concentration ($\mu\text{g L}^{-1}$) for triplicate microcosm samples at time zero and 7, 17, 28, and 42 days of incubation at 5° C.

	Day	Replicate	DOSS ug L-1	EHSS ug L-1	Σ Tween 80 Tween 85 ug L-1	Span 80 (ug/L) ug L-1
Corexit-Only	0	1	480	10	766	147
		2	550	11	898	142
		3	570	10	776	155
		Mean	533	10	813	148
		SD	47	0.49	73.5	6.6
		95 CI	117.4	1.2	52.7	4.7
	7	1	810	10	<LOD	<LOD
		2	650	10	<LOD	<LOD
		3	860	10	<LOD	<LOD
		Mean	770	10	NC	NC
		SD	110	0.14	NC	NC
		95 CI	272.5	0.3	NC	NC
	17	1	540	11	<LOD	<LOD
		2	470	11	<LOD	<LOD
		3	540	10	<LOD	<LOD
		Mean	520	11	NC	NC
		SD	40	0.40	NC	NC
		95 CI	100.4	1.0	NC	NC
	28	1	490	11	<LOD	<LOD
		2	500	11	<LOD	<LOD
		3	470	12	<LOD	<LOD
		Mean	490	11	NC	NC
		SD	15	0.31	NC	NC
		95 CI	37.9	0.8	NC	NC
	42	1	1560	15	<LOD	<LOD
		2	990	9	<LOD	<LOD
		3	150	14	<LOD	<LOD
		Mean	900	13	NC	NC
		SD	710	3.4	NC	NC
		95 CI	1763.9	8.5	NC	NC

Table A1.1 Continued.

	Day	Replicate	DOSS	EHSS	Σ Tween 80	Tween 85	Span 80 (ug/L)
			ug L-1	ug L-1	ug L-1		ug L-1
CEWAF	0	1	18	20	<LOD		<LOD
		2	21	20	<LOD		<LOD
		3	17	18	<LOD		<LOD
		Mean	19	19	NC		NC
		SD	2.1	1.6	NC		NC
		95 CI	1.5	1.2	NC		NC
	7	1	17	18	<LOD		<LOD
		2	15	20	<LOD		<LOD
		3	17	19	<LOD		<LOD
		Mean	16	19	NC		NC
		SD	0.8	0.6	NC		NC
		95 CI	2.1	1.6	NC		NC
	17	1	13	18	<LOD		<LOD
		2	19	23	<LOD		<LOD
		3	17	23	<LOD		<LOD
		Mean	17	21	NC		NC
		SD	3.2	2.6	NC		NC
		95 CI	2.3	1.8	NC		NC
	28	1	15	21	<LOD		<LOD
		2	12	19	<LOD		<LOD
		3	12	19	<LOD		<LOD
		Mean	13	20	NC		NC
		SD	1.3	1.3	NC		NC
		95 CI	3.3	3.2	NC		NC
	42	1	13	19	<LOD		<LOD
		2	10	21	<LOD		<LOD
		3	14	19	<LOD		<LOD
		Mean	12	20	NC		NC
		SD	1.7	1.3	NC		NC
		95 CI	4.2	3.3	NC		NC

Table A1.1 Continued.

	Day	Replicate	DOSS ug L-1	EHSS ug L-1	Σ Tween 80 Tween 85 ug L-1	Span 80 (ug/L) ug L-1
CEWAF + N	0	1	18	19	<LOD	<LOD
		2	19	17	<LOD	<LOD
		3	19	20	<LOD	<LOD
		Mean	18	19	NC	NC
		SD	0.32	1.1	NC	NC
		95 CI	0.8	2.8	NC	NC
	7	1	16	18	<LOD	<LOD
		2	14	20	<LOD	<LOD
		3	18	20	<LOD	<LOD
		Mean	16	19	NC	NC
		SD	2.1	1.0	NC	NC
		95 CI	5.1	2.4	NC	NC
	42	1	9	19	<LOD	<LOD
		2	11	18	<LOD	<LOD
		3	12	30	<LOD	<LOD
		Mean	11	22	NC	NC
		SD	1.3	6.8	NC	NC
		95 CI	3.3	16.8	NC	NC

Table A1.2. DOSS, EHSS and Tween 80 and Tween 85 concentration ($\mu\text{g L}^{-1}$) for triplicate abiotic control microcosm samples prior to (pre) and after (post) double pasteurization and at 7 and 39 days of incubation at 8° C.

Day	Replicate	DOSS $\mu\text{g L}^{-1}$	EHSS $\mu\text{g L}^{-1}$	Σ Tween 80 Tween 85 $\mu\text{g L}^{-1}$
0 Pre	1	2100	17	3500
	2	2000	19	3400
	3	2100	15	3400
	Mean	2100	17	3400
	SD	60	2.3	58
	95 CI	140	5.6	140
0 Post	1	2100	19	3600
	2	2600	17	4200
	3	2300	14	3900
	Mean	2300	17	3900
	SD	250	2.1	300.0
	95 CI	620	5.2	745.3
7	1	2200	11	3600
	2	2000	10	3600
	3	2000	16	3800
	Mean	2100	12	3700
	SD	120	3.4	120
	95 CI	290	8.6	290
39	1	2200	18	3100
	2	2100	20	3100
	3	2000	21	3500
	Mean	2100	20	3200
	SD	100	1.2	230
	95 CI	250	3.0	570

Table A1.3. Mass (g) of DOSS, EHSS, Span 80 and Tween 80 and Tween 85 in 100 g of Corexit 9500 and the surfactant to EHSS ratio.

	Mass (g)	EHSS ratio
DOSS	18	64
EHSS	0.28	NA
Tween 80	22.6	81
Span 80	4.4	16

Table A14. The surfactant to EHSS ratio for DOSS, EHSS, Span 80, and Tween 80 and Tween 85. for triplicate Corexit –only, CEWAF and CEWAF + nutrients microcosm and abiotic controls at time zero.

Treatment	Replicate	DOSS ug L-1	EHSS ug L-1	Σ Tween 80 Tween 85 ug L-1	Span 80 (ug/L) ug L-1
C9500	1	50	NA	80	15
	2	52	NA	86	14
	3	59	NA	80	16
	Mean	54	NC	82	15
	SD	4.4	NC	3.3	1.3
	95 CI	11	NC	2.3	0.90
CEWAF	1	0.90	NA	<LOD	<LOD
	2	1.0	NA	<LOD	<LOD
	3	0.95	NA	<LOD	<LOD
	Mean	1.0	NC	NC	NC
	SD	0.06	NC	NC	NC
	95 CI	0.15	NC	NC	NC
CEWAF+ N	1	0.94	NA	<LOD	<LOD
	2	1.1	NA	<LOD	<LOD
	3	0.95	NA	<LOD	<LOD
	Mean	1.0	NC	NC	NC
	SD	0.07	NC	NC	NC
	95 CI	0.18	NC	NC	NC
Abiotic 8° C Post	1	110	19	200	NA*
	2	150	17	250	NA*
	3	160	14	270	NA*
	Mean	140	17	240	NC
	SD	25	2.1	39	NC
	95 CI	61	5.2	96	NC
Abiotic 25° C	1	93	NA	120	NA*
	2	68	NA	64	NA*
	3	84	NA	79	NA*
	Mean	81	NC	87	NC
	SD	13	NC	29	NC
	95 CI	32	NC	73	NC

Table A1.5. The concentration of EHSS ($\mu\text{g L}^{-1}$) calculated using a bulk dilution factor, the measured EHSS concentration ($\mu\text{g L}^{-1}$), and the fraction of EHSS incorporated into the aqueous phase for the Corexit-only, CEWAF ($\pm$ nutrients) treatments, abiotic controls, and additional Corexit-only and CEWAF ($\pm$ nutrients) solutions.

Treatment	Calculated EHSS $\mu\text{g L}^{-1}$	Measured EHSS $\mu\text{g L}^{-1}$	Percent EHSS %
C9500	42	10	24
CEWAF	74	19	26
CEWAF+ N	75	18	24
Abiotic 8° C Post	700	17	2.4
Abiotic 25° C	17.5	8.0	46
C9500 2	700	26	3.7
CEWAF 2	350	24.00	6.9
CEWAF+ N 2	350	24.0	6.9

Table A1.6. DOSS and EHSS concentration ($\mu\text{g L}^{-1}$) for additional triplicate t time zero Corexit-only and CEWAF ($\pm$ nutrients) initial solutions.

Treatment	Replicate	DOSS $\mu\text{g L}^{-1}$	EHSS $\mu\text{g L}^{-1}$	Σ Tween 80 Tween 85 $\mu\text{g L}^{-1}$
C9500	1	2200	25	2800
	2	2300	23	2900
	3	2300	29	2900
	Mean	2300	26	2900
	SD	58	2.9	58
	95 CI	140	7.1	140
CEWAF	1	21	23	<LOD
	2	27	26	<LOD
	3	22	22	<LOD
	Mean	23	24	NC
	SD	3.3	1.9	NC
	95 CI	8.2	4.6	NC
CEWAF+ N	1	24	26	<LOD
	2	25	25	<LOD
	3	26	21	<LOD
	Mean	25	24	NC
	SD	1.0	2.7	NC
	95 CI	2.5	6.8	NC

Table A1.7. DOSS, EHSS and Tween 80 and Tween 85 concentration ($\mu\text{g L}^{-1}$) for triplicate abiotic control microcosm at time zero, 7 and 24 days of incubation at 25° C.

Day	Replicate	DOSS ug L-1	EHSS ug L-1	Σ Tween 80 Tween 85 ug L-1
0	1	530	5.7	680
	2	670	9.9	630
	3	640	7.6	600
	Mean	610	8	640
	SD	74	2.1	40
	95 CI	180	5.2	100
7	1	620	8.3	800
	2	680	8.0	650
	3	720	12	540
	Mean	670	9.4	660
	SD	50	2.2	130.0
	95 CI	130.0	5.4	320.0
24	1	630	8.8	1100
	2	730	13	1500
	3	750	19	1100
	Mean	700	14	1200
	SD	64	5.4	230.0
	95 CI	160	13	570

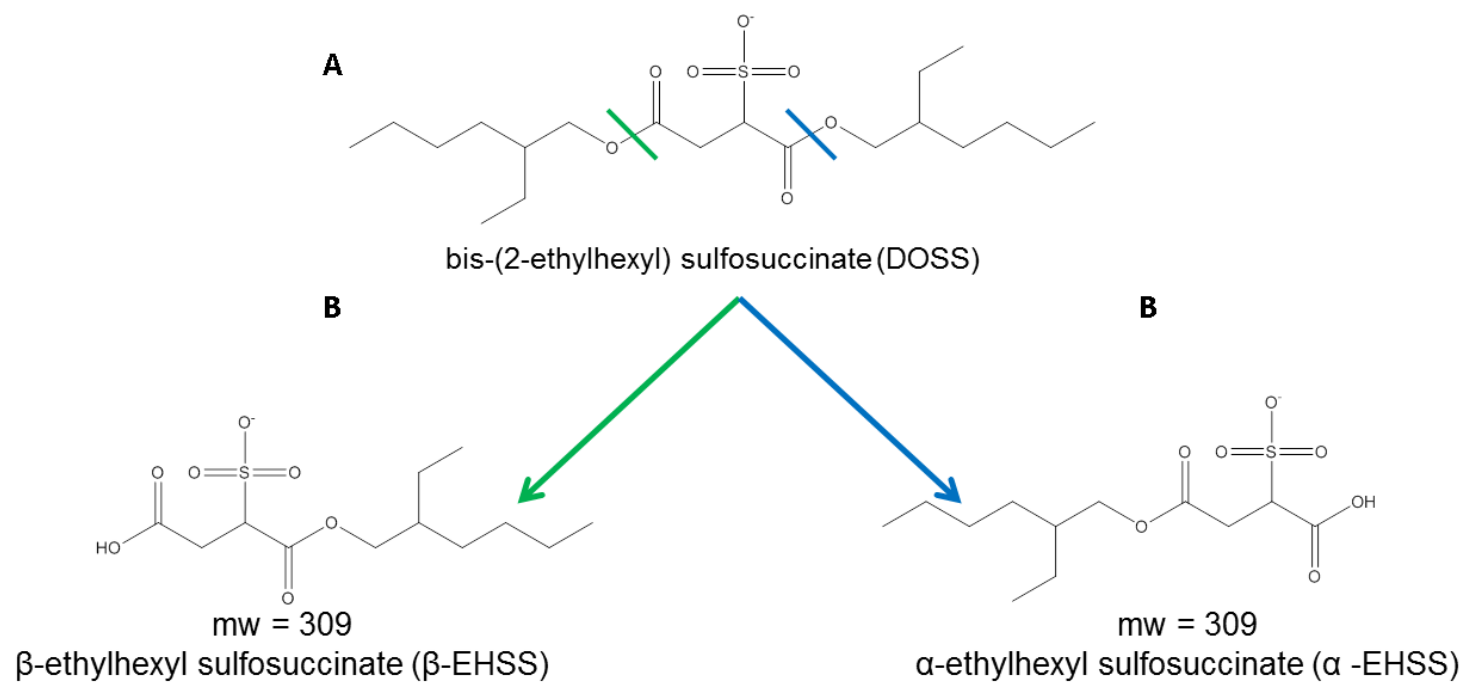


Figure A1.1. Chemical structure of DOSS (Bis-(2-ethylhexyl)sulfosuccinate) (**A**) and EHSS (α/β -ethylhexylsulfosuccinate) (**B**).

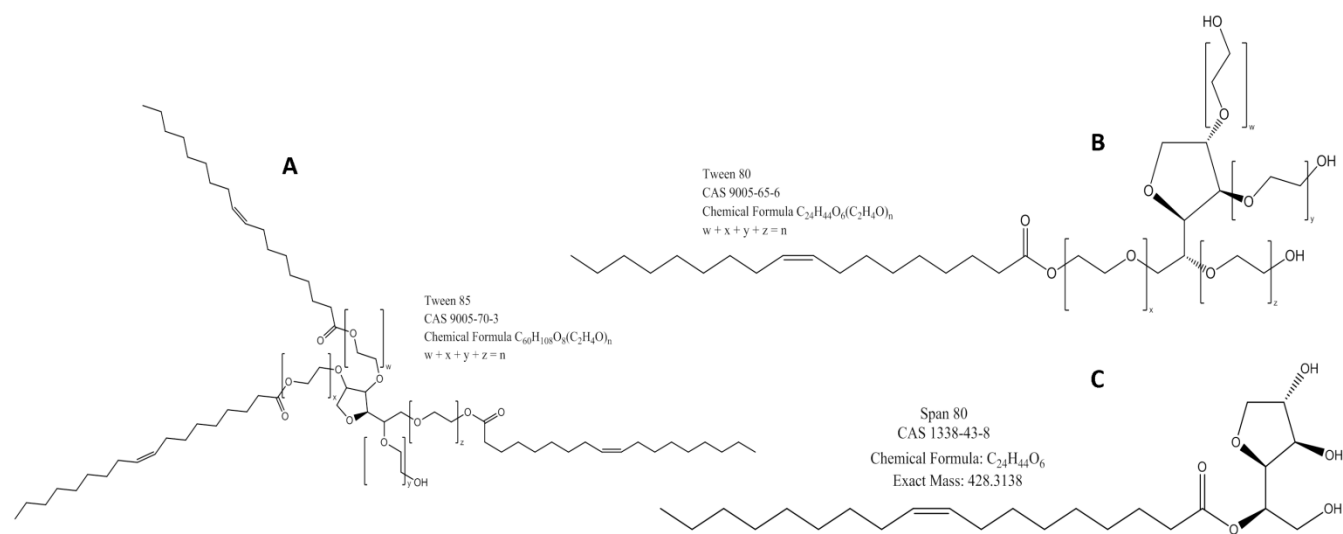


Figure A1.2. Chemical Structure and Tween 85 (A), Tween 80(B), and Span 80 (C).

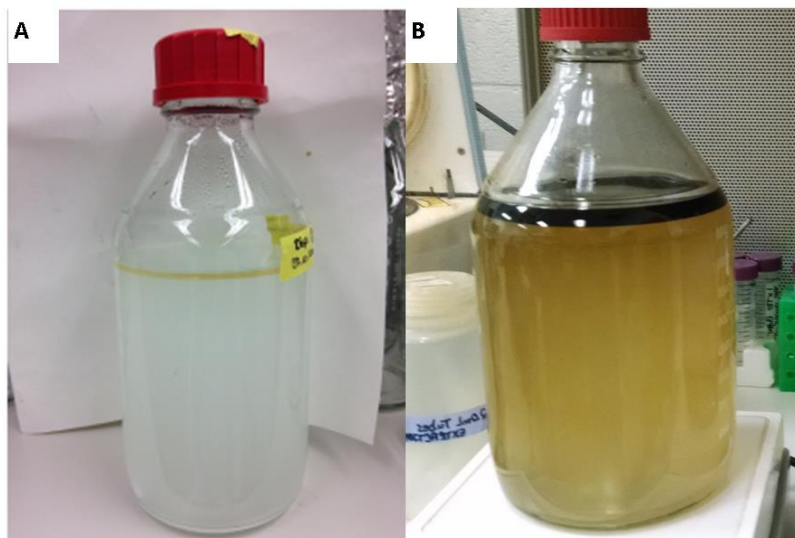


Figure A1.3. Corexit-only (A) and CEWAF (B) after 48 h of stirring.

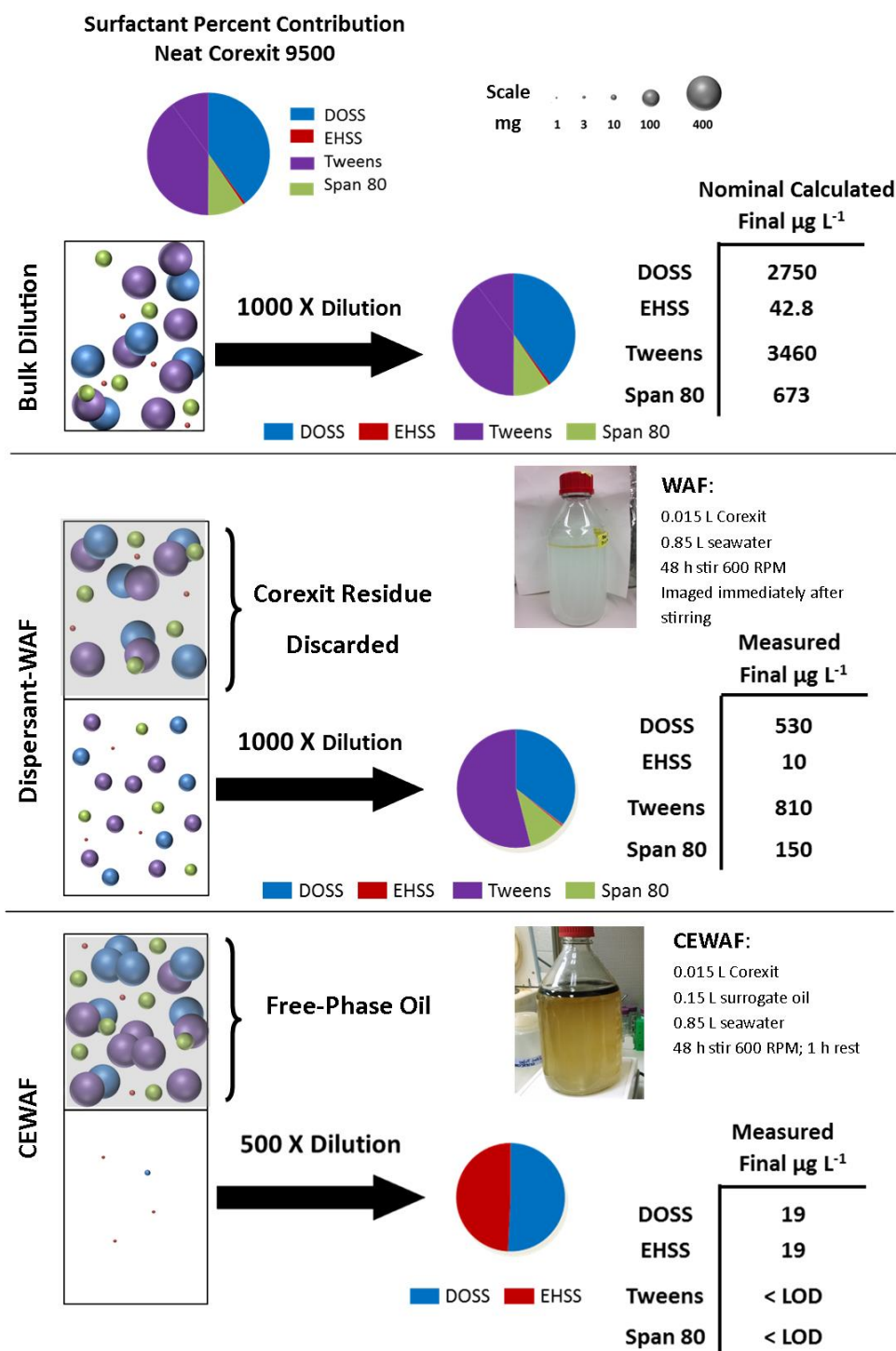


Figure A1.4. Distribution of the primary surfactants in COREXIT and their expected distribution upon 1000X dilution (top panel) versus observed distribution in the Corexit-only solution (middle panel) and the CEWAF solution (lower panel).

Appendix 2 – Chapter 3 Supporting Information: Selective Quantification of DOSS in Marine Sediment and Sediment-Trap Solids by LC-QTOF-MS

A2.1 Liquid Chromatography.

An Agilent Poroshell 120 EC-C18 guard column (4.6 mm ID x 5 mm length x 2.7 μm particle size) that was placed in-line with an Agilent XDB-C18 analytical column (4.6 mm ID x 20 mm length x 3.57 μm particle size). A 900 μL extended seat capillary was inserted between the needle seat and the six port valve in the auto sampler. Mobile phase A consisted of 0.5 mM NH_4OAc in water, mobile phase B was 100 percent MeOH, and mobile phase C was 10 mM NH_4OAc in methanol. The initial mobile phase was 5% B at an initial flow rate of 2.5 mL min^{-1} . This condition was held for 2.1 min, increased to 70 % by 2.5 min, increased to 85% by 3 min, and increased to 100% by 3.35 min. At 3 min, the flow rate was reduced to 1 mL min^{-1} to increase the ionization efficiency of DOSS during elution, and at 4.5 min, the flow rate was increased back to 2.5 mL min^{-1} . At 6 min, 100% C was rapidly introduced and the flow rate was increased to 4.25 mL min^{-1} and then held for 2 min to regenerate the SAX guard columns. The system was then flushed with 100% mobile phase B for 1 min. Initial mobile phase conditions were gradually reintroduced between 9 and 10 min and held for an additional 1.5 min before beginning the next analysis. Using the external divert valve, flow from the analytical column was diverted to waste for the first 2.1 min of the gradient to prevent the injection of non-volatile salts into the MS source. The flow was again diverted to waste at 4.5 min and held in that position for the remainder of the gradient.

Table A2.1. DOSS concentration ($\mu\text{g kg}^{-1}$) calculated for extraction blanks.

Extract	Calculated Concentration ($\mu\text{g kg}^{-1}$)
Extraction blank 1	0.10
Extraction blank 2	s/n < 3
Extraction blank 3	s/n < 3
Extraction blank 4	0.08
Extraction blank 5	0.36
Extraction blank 6	0.07
Extraction blank 7	0.05
Extraction blank 8	0.02
Extraction blank 9	s/n < 3
Extraction blank 10	0.05
Extraction blank 11	0.61
Extraction blank 12	0.08
Extraction blank 13	0.15
Extraction blank 14	0.14
Extraction blank 15	s/n < 3
Extraction blank 16	s/n < 3
Extraction blank 17	s/n < 3
Extraction blank 18	s/n < 3

Table A2.2. The effect of freeze drying and storage on DOSS concentrations . Mean DOSS concentration ($\mu\text{g kg}^{-1}$), and 95% confidence interval, and RSD calculated for replicate extracts of a single sediment sample containing environmentally-incurred DOSS. The sediment sample was split into subsamples and either freeze-dried or not freeze-dried. Sediment not freeze-dried was extracted in triplicate and analyzed by LS-MS/MS during the initial method optimization. Sediment that was freeze-dried was extracted in quadruplicate and analyzed by LC-QTOF-MS and separate extracts of freeze-dried sediment were analyzed again by LC-QTOF-MS; 12 months later.

Analyte	Not Freeze-Dried	Freeze-Dried	Freeze-Dried 12 months
	(n = 3)	(n = 4)	(n = 4)
DOSS	$1.8 \pm 1.0 \mu\text{g kg}^{-1}$ (22%)	$1.4 \pm 0.4 \mu\text{g kg}^{-1}$ (16%)	$1.1 \pm 0.2 \mu\text{g kg}^{-1}$ (8.7%)

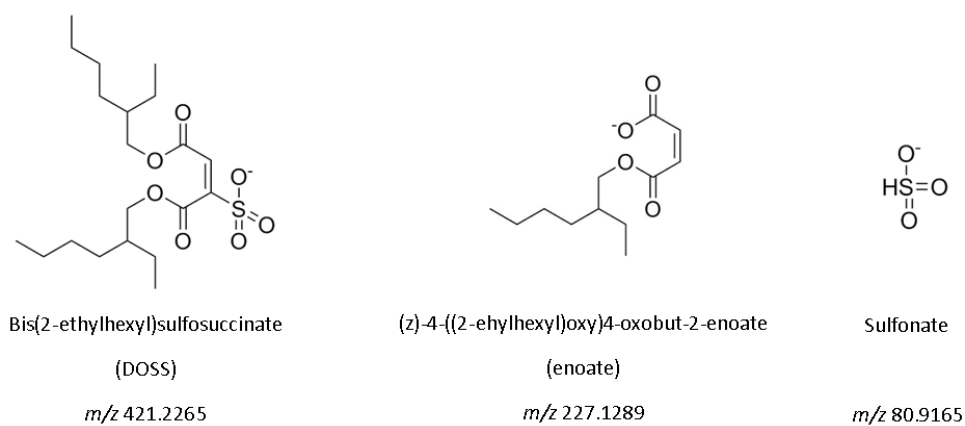


Figure A2.1. Structure and molecular weight of DOSS (*m/z* 421.2265) and its characteristic fragment ions including (z)-4-((2ethylhexyl)oxy)4-oxobut-2-enoate (*m/z* 227.1289) and sulfonate (*m/z* 80.9165).

Appendix 3 – Chapter 4 Supporting Information: SPATIAL AND TEMPORAL TRENDS IN DOSS IN GULF OF MEXICO DEEP-SEA SEDIMENTS: 2010 AND 2015

A3.1 Chemicals and standards. Solid (98.1%) bis-(2-ethylhexyl) sodium sulfosuccinate (DOSS) and deuterated DOSS ($^2\text{H}_{34}$ -DOSS) was obtained from Sigma Aldrich (Saint Louis, MO, USA). Parent stock standards and analytical standards were prepared as described previously (Perkins et al. 2016). Analytical standards were prepared in MeOH and analyzed within eight h. All glassware was combusted at 400 °C for 12 h prior to use. MS-grade methanol (MeOH) was purchased from Fisher Scientific (Pittsburg, PA, USA). Laboratory 18-M Ω , deionized (DI) water was obtained by an in-house Millipore Synergy unit with an LC-Pak polisher (EMD Millipore Corp, Billerica, MA, USA). High purity ammonium acetate was purchased from Sigma Aldrich. Acid-washed sand was purchased from Macron Fine Chemicals (Center Valley, PA, USA).

A3.2 Liquid Chromatography. An Agilent Poroshell 120 EC-C18 guard column (4.6 mm ID x 5 mm length x 2.7 μm particle size) that was placed in-line with an Agilent XDB-C18 analytical column (4.6 mm IDx20 mm lengthx3.57 μm particle size). A 900 μL extended seat capillary was inserted between the needle seat and the six port valve in the auto sampler. Mobile phase A consisted of 0.5 mM NH_4OAc in water, mobile phase B was 100 percent MeOH, and mobile phase C was 10 mM NH_4OAc in methanol. The initial mobile phase was 5% B at an initial flow rate of 2.5 mL min^{-1} . This condition was held for 2.1 min, increased to 70 % by 2.5 min, increased to 85% by 3 min, and increased to 100% by 3.35 min. At 3 min, the flow rate was reduced to 1 mL min^{-1} to increase the ionization efficiency of DOSS during elution, and at 4.5 min, the flow rate was increased back to 2.5 mL min^{-1} . At 6 min, 100% C was rapidly introduced and the flow rate was increased to 4.25 mL min^{-1} and then held for 2 min to regenerate the SAX guard columns. The system was then flushed with 100% mobile phase B for 1 min. Initial mobile phase conditions were gradually reintroduced between 9 and 10 min and held for an additional 1.5 min before beginning the next analysis. Using the external divert valve, flow from the

analytical column was diverted to waste for the first 2.1 min of the gradient to prevent the injection of non-volatile salts into the MS source. The flow was again diverted to waste at 4.5 min and held in that position for the remainder of the gradient.

LC-QTOF-MS Calibration and Quality Control. Calibration curves for DOSS were prepared on each day of analysis and each consisted of five calibration standards that spanned from 0.27 to 3.6 ng injected DOSS. Extracts with DOSS concentrations greater than 3.6 ng were re-extracted and reanalyzed with a greater range of calibration (0.27-28 ng). The $^2\text{H}_{34}$ -DOSS (internal standard) was added to all calibration standards, such that 0.56 ng of $^2\text{H}_{34}$ -DOSS was injected. Calibration curves were 1/x weighted and required to give an $R^2 > 0.99$ with deviation <20% for all calibration points. The uncertainties about the reported DOSS concentrations were determined from spike and recovery experiments as described previously.(16)

Check standards were used for quality control purposes and consisted of 900 ng L^{-1} DOSS, which is the equivalent of 0.27 ng DOSS was injected. Check standards were run every eight samples, were required to have a calculated concentration within 20% of the expected value, and were used to verify the continuing performance of the instrument. To continually check for DOSS contamination, blank methanol and an extraction blank were run at least every eight samples along with the check standards.

High Resolution Quadrupole Time of Flight (QTOF) Mass Spectrometry. QTOF analysis was performed on a TripleTOF ABSciex 5600 (Framingham, MA, USA) instrument equipped with an electrospray interface and operated in negative ionization mode at a resolution greater than 20,000 FWHH. In full scan mode, data was collected in 800 cycles with a period cycle time of 450 ms, a pulser frequency of 18.079 kHz, and an accumulation time of 0.1 ms. The curtain, nebulizer (gas 1), and drying (gas 2) gases were 30, 50, and 40 psi, respectively. The ion spray voltage was set at -4500 V with a temperature of 550°C, a collision energy of -10 V, and a declustering potential of -80 V. The starting and ending masses were 70 and 1000, respectively. In QTOF MS/MS mode, the instrument was operated under the same conditions, except with a collision energy of -27 V and spread of 0.0, an ion release delay of 66.63, and an ion release width of 24.92.

The instrument was calibrated with the APCI Negative Calibration Solution (ABSciex) before each extract was injected.

The instrument was run concurrently in full scan mode to capture the molecular ions of DOSS (m/z 421.2265) and $^2\text{H}_{34}$ -DOSS (m/z 455.4400) and in QTOF mode to collect MS/MS fragment data for DOSS and $^2\text{H}_{34}$ -DOSS. Quantification for LC-QTOF-MS was performed using Multiquant (version 2.1). Molecular ions for DOSS (m/z 421.2265 $\pm$ 15 ppm) and the $^2\text{H}_{34}$ -DOSS internal standard (m/z 455.4400 $\pm$ 15 ppm) were extracted for quantification. The fragment ions of DOSS (m/z 227.1278 and 80.9156 $\pm$ 15 ppm), and $^2\text{H}_{34}$ -DOSS (m/z 244.2356 and 80.9156 $\pm$ 15 ppm) were used for confirmation.

A3.5 NRDA Dataset. This data set is publically through the NRDA Deepwater Horizon Public Diver Explorer at <https://dwhdiver.orr.noaa.gov/explore-the-data>. DOSS data was last accessed on 12/15/2016 and can be downloaded under sediment chemistry exports for the “Deepwater ROV Sampling to Assess Potential Impacts to Hardbottom Coral Communities and Associates from the Deepwater Horizon Oil Spill” and the “Deepwater Sediment Sampling to Assess Potential Post-Spill Benthic Impacts from the Deepwater Horizon Oil Spill” gulf work plans.

Table A3.1. Year collected, DOSS concentration ($\mu\text{g kg}^{-1}$), radial distance from the DWH (km), latitude and longitude (decimal degrees), and the depth interval (cm) for surficial sediments collected between 2010 and 2015 where DOSS was not overserved above the LLOQ ($0.23 \mu\text{g kg}^{-1}$).

Year	DOSS Concentration ($\mu\text{g kg}^{-1}$)	Radial Distance (km)	Latitude	Longitude	Depth (cm)
2010	<LLOQ	8.3	28.681833	-88.309733	0-2.5
2010	<LLOQ	47	28.908017	-87.922333	0-3
2010	<LLOQ	49	28.536667	-87.939667	0-3
2010	<LLOQ	68	28.852	-89.057833	0-3
2010	<LLOQ	90	29.046467	-87.50415	0-1
2011	<LLOQ	18	28.855167	-88.491333	OF
2012	<LLOQ	5.0	28.712	-88.324	0-3
2012	<LLOQ	7.0	28.67505	-88.369433	0-3
2012	<LLOQ	7.0	28.67505	-88.369433	OF
2012	<LLOQ	63	28.939283	-88.970167	0-3
2012	<LLOQ	65	29.32475	-88.386767	0-3
2012	<LLOQ	140	27.555	-88.766667	0-3
2013	<LLOQ	16	28.639917	-88.486767	0-3
2013	<LLOQ	18	28.856167	-88.489667	0-5
2013	<LLOQ	280	27.365767	-90.564117	0-3
2015	<LLOQ	4.5	28.697833	-88.365167	OF
2015	<LLOQ	4.5	28.699717	-88.381133	OF
2015	<LLOQ	29	28.637383	-88.637383	0-5
2015	<LOD	250	27.365333	-90.365333	0-5
2015	<LLOQ	310	27.209333	-91.014667	0-5
2015	<LLOQ	310	27.209333	-91.014667	OF
2015	<LLOQ	310	27.209	-91.014667	OF
2015	<LLOQ	320	27.208667	-91.015167	0-5
2015	<LLOQ	320	27.758833	-91.43405	0-5
2015	<LOD	330	27.783133	-91.50735	0-5
2015	<LLOQ	350	27.758833	-91.758833	0-5

Table A3.2. Year collected, DOSS concentration ($\mu\text{g kg}^{-1}$), radial distance from the DWH (km), latitude and longitude (decimal degrees), and the depth interval (cm) for surficial sediments collected on the Holiday Chouest cruise. LLOQ = $5 \mu\text{g kg}^{-1}$.

Year	DOSS Concentration ($\mu\text{g kg}^{-1}$)	Radial Distance (km)	Latitude	Longitude	Depth (cm)
2011	<LLOQ	4.9	28.69606	-88.346325	0-1
2011	6.1	6.4	28.68222	-88.345014	0-1
2011	<LLOQ	6.5	28.68136	-88.34402	0-1
2011	9.3	13	28.67222	-88.476382	0-1
2011	<LLOQ	13	28.67213	-88.476427	0-1
2011	<LLOQ	18	28.85262	-88.492557	0-1
2011	<LLOQ	18	28.85595	-88.493617	0-1
2011	<LLOQ	22	28.63381	-88.169696	0-1
2011	<LLOQ	22	28.63359	-88.169516	0-1
2011	<LLOQ	27	28.78727	-88.634709	0-1
2011	<LLOQ	27	28.93538	-88.202652	0-1
2011	<LLOQ	27	28.93541	-88.201376	0-1
2011	<LLOQ	37	29.06942	-88.377483	0-1
2011	<LLOQ	37	29.06993	-88.377591	0-1
2011	<LLOQ	55	28.4855	-88.850837	0-1
2011	<LLOQ	59	29.16498	-88.011441	0-1
2011	<LLOQ	180	27.58663	-89.704054	0-1
2011	<LLOQ	180	27.5865	-89.704481	0-1

Table A3.3. Year collected, DOSS concentration ($\mu\text{g kg}^{-1}$), radial distance from the DWH (km), latitude and longitude (decimal degrees), and the depth interval (cm) for surficial sediments collected by the NRDA program on the Sarah Bordelon cruise. LLOQ = $5 \mu\text{g kg}^{-1}$.

Year	DOSS Concentration ($\mu\text{g kg}^{-1}$)	Radial Distance (km)	Latitude	Longitude	Depth (cm)
2011	20	0.42	28.73995	-88.36815	0-1
2011	25	0.43	28.73994	-88.36821	0-1
2011	79	0.43	28.73438	-88.36243	0-1
2011	27	0.44	28.73432	-88.36239	0-1
2011	54	0.44	28.73433	-88.36237	0-1
2011	20	0.46	28.73455	-88.37008	0-1
2011	41	0.47	28.74047	-88.36805	0-1
2011	24	0.48	28.73443	-88.37021	0-1
2011	19	0.49	28.73438	-88.37028	0-1
2011	20	0.70	28.74228	-88.36273	0-1
2011	24	0.70	28.74226	-88.36268	0-1
2011	23	0.71	28.74232	-88.3627	0-1
2011	6100	0.76	28.74211	-88.37066	0-1
2011	32	0.78	28.7422	-88.37094	0-1
2011	27	0.80	28.7426	-88.37053	0-1
2011	20	0.86	28.73164	-88.3592	0-1
2011	60	0.88	28.73211	-88.35862	0-1
2011	84	0.91	28.73128	-88.35887	0-1
2011	20	1.1	28.74479	-88.35983	0-1
2011	23	1.1	28.74482	-88.35988	0-1
2011	20	1.1	28.74474	-88.35973	0-1
2011	11	1.2	28.73228	-88.37673	0-1
2011	25	1.2	28.73158	-88.37651	0-1
2011	20	1.2	28.73155	-88.37657	0-1
2011	14	1.2	28.74443	-88.37438	0-1
2011	20	1.2	28.7445	-88.37447	0-1
2011	10	1.2	28.74464	-88.37441	0-1
2011	5.8	2.8	28.72013	-88.34459	0-1
2011	20	2.8	28.71994	-88.34464	0-1
2011	<LLOQ	2.8	28.71987	-88.34469	0-1
2011	33	2.9	28.72002	-88.38837	0-1
2011	58	2.9	28.72	-88.38894	0-1
2011	15	2.9	28.71995	-88.38897	0-1
2011	9.9	3.0	28.73872	-88.33581	0-1

Table A3.3. Continued.

Year	DOSS Concentration ($\mu\text{g kg}^{-1}$)	Radial Distance (km)	Latitude	Longitude	Depth (cm)
2011	6.3	3.0	28.73911	-88.33568	0-1
2011	27	3.0	28.70987	-88.36599	0-1
2011	12	3.0	28.70979	-88.36671	0-1
2011	20	3.0	28.73879	-88.33541	0-1
2011	68	3.0	28.7096	-88.36661	0-1
2011	20	3.1	28.73798	-88.39733	0-1
2011	24	3.1	28.73805	-88.39751	0-1
2011	22	3.1	28.73825	-88.39755	0-1
2011	6.9	3.2	28.75798	-88.34446	0-1
2011	20	3.2	28.75689	-88.38887	0-1
2011	20	3.2	28.76516	-88.36656	0-1
2011	20	3.2	28.75774	-88.34408	0-1
2011	13	3.2	28.76517	-88.36664	0-1
2011	9.8	3.2	28.75786	-88.3442	0-1
2011	8.1	3.2	28.76519	-88.36677	0-1
2011	<LLOQ	3.2	28.75732	-88.38859	0-1
2011	<LLOQ	4.8	28.69686	-88.38516	0-1
2011	7.1	4.8	28.69659	-88.38511	0-1
2011	25	4.8	28.69643	-88.38524	0-1
2011	9.4	4.9	28.70546	-88.40203	0-1
2011	20	4.9	28.70545	-88.4022	0-1
2011	12	5.0	28.70481	-88.40185	0-1
2011	19	5.0	28.73015	-88.41697	0-1
2011	20	5.1	28.73002	-88.41751	0-1
2011	9	5.1	28.73018	-88.41756	0-1
2011	47	6.4	28.79245	-88.34849	0-1
2011	17	6.4	28.79248	-88.34857	0-1
2011	20	6.4	28.79255	-88.34862	0-1
2011	20	7.1	28.67298	-88.36899	0-1
2011	39	7.1	28.67271	-88.36882	0-1
2011	9.7	7.1	28.67267	-88.3686	0-1
2011	5.7	7.4	28.68808	-88.41834	0-1
2011	9.3	7.4	28.68793	-88.41833	0-1
2011	25	7.5	28.68772	-88.41852	0-1

Table A3.3. Continued.

Year	DOSS Concentration ($\mu\text{g kg}^{-1}$)	Radial Distance (km)	Latitude	Longitude	Depth (cm)
2011	5.4	9.9	28.66803	-88.43009	0-1
2011	6.6	9.9	28.66782	-88.43004	0-1
2011	29	9.9	28.66761	-88.43005	0-1
2011	11	10	28.78467	-88.4538	0-1
2011	6	10	28.78488	-88.45369	0-1
2011	24	10	28.78455	-88.45405	0-1
2011	37	15	28.63902	-88.47105	0-1
2011	<LLOQ	15	28.63909	-88.47122	0-1
2011	6.5	15	28.63916	-88.47131	0-1
2011	83	20	28.7746	-88.16809	0-1
2011	5.7	20	28.77458	-88.16793	0-1
2011	5.4	20	28.77466	-88.16755	0-1
2011	<LLOQ	21	28.80296	-88.56454	0-1
2011	20	21	28.80303	-88.56485	0-1
2011	48	21	28.80312	-88.56505	0-1
2011	10	22	28.75743	-88.58863	0-1
2011	8.8	29	28.55159	-88.57892	0-1
2011	<LLOQ	29	28.55162	-88.57906	0-1
2011	20	29	28.55135	-88.5793	0-1
2011	5.8	34	28.51423	-88.60058	0-1
2011	20	34	28.5139	-88.60032	0-1
2011	6.6	34	28.514	-88.60055	0-1
2011	<LLOQ	49	28.41577	-88.70413	0-1
2011	11	49	28.41557	-88.70421	0-1
2011	20	49	28.41533	-88.70412	0-1
2011	5.4	51	28.98893	-87.93463	0-1
2011	<LLOQ	51	28.98911	-87.93466	0-1
2011	20	51	28.98889	-87.93434	0-1
2011	20	62	29.10662	-87.88882	0-1
2011	6.8	62	29.10682	-87.88892	0-1
2011	10	62	29.1068	-87.88847	0-1
2011	16	62	28.55727	-87.7607	0-1
2011	5.3	62	28.55687	-87.76033	0-1
2011	21	62	28.55672	-87.76028	0-1

Table A3.3. Continued.

Year	DOSS Concentration ($\mu\text{g kg}^{-1}$)	Radial Distance (km)	Latitude	Longitude	Depth (cm)
2011	27	71	28.91842	-87.67225	0-1
2011	6	71	28.91857	-87.67216	0-1
2011	6.2	71	28.91859	-87.672	0-1
2011	5.9	76	28.2658	-88.92334	0-1
2011	9.9	76	28.26536	-88.92321	0-1
2011	20	76	28.2653	-88.92335	0-1
2011	5.4	120	28.21866	-89.49174	0-1
2011	16	120	28.21865	-89.49184	0-1
2011	20	120	28.21852	-89.49195	0-1
2011	17	130	27.82841	-89.16478	0-1
2011	21	130	27.82806	-89.16479	0-1
2011	20	130	27.82796	-89.16469	0-1

Appendix 4 – Chapter 5 Supporting Information: EJECTION OF SURFACTANTS FROM COREXIT 9500A/9527A AND ALKANES FROM OIL INTO THE ATMOSPHERE VIA AEROSOLIZATION FROM THE SEA SURFACE

A4.1 Aerosolization Reactor. The reactor consisting of 11.5-cm wide, 1.5-m long glass tube that was filled with about 5 L of either NaCl solution, 35 % (w/w), or seawater. The reactor incorporates an annular shear sparger at the bottom to produce abundant small-sized bubbles (0.25 ± 0.04 mm).² The produced bubbles in sparger are sheared off by a liquid shear flow, induced by a peristaltic pump (Masterflex I/P 77600-62, Thermo Fisher Scientific, Barrington, IL, USA). After rising to the surface, bubbles burst and produce droplets. A ring shaped air-lift inside the reactor facilitates lifting and drying the ejected droplets and vapors to the top part of the reactor, where they are collected. The operating parameters of the reactor including bubble flow, shear flow, and air-lift flow were set at 5 L min⁻¹, 6.4 L min⁻¹, and 40 L min⁻¹, respectively.

A4.2 LC-MS/MS Surfactant Analysis. Chromatographic separations were performed on an Agilent 1100 (Agilent Technologies, Inc., Santa Clara, CA) as described in Place et al.³ with minor modification. Briefly, an Agilent Proshell 120 EC-C18 guard column (4.6 mm ID_5 mm length_2.7 μ m particle size) was placed in front of an Agilent XDB-C18 (DOSS) or an Agilent XDB-C8 (Tween 80 and Tween 85) analytical column (4.6 mm ID_20 mm length_3.5 μ m particle size; C8 and C18). Also, during the analysis of the nonionic surfactants, the mobile phase was directed through an Agilent thermostatted column compartment (G1316A) and heated to 40 °C.

Additionally, the initial flow rate was increased to 1 mL min⁻¹ during sample loading and washing of the non-volatile salts from the column (first 5.6 minutes) without degrading the peak shape or percent recovery of the analytes of interest. The gradient was further modified such that the 97.5 % acetonitrile was held for 3 min before returning to 5 % acetonitrile for 6 min. The flow rate was 1 mL min⁻¹ for the first 5.6 min, 0.5 mL min⁻¹ for

5 min, and 1.0 mL min⁻¹ for 6 minutes. The timing of the main-pass-by-pass valve switching and divert valve switching, as described by Place et al.³ was adjusted to reflect changes in the flow rate and gradient. These chromatographic changes were made to achieve better peak symmetry and to significantly shorten the run time of the analysis.

Tandem quadrupole mass spectrometric analysis was performed as described in Place et al.³, except that the analysis of Span 80 was not acquired concurrently with that of Tween 80 and Tween 85. For whole Corexit samples, a separate analysis was performed for Span 80 (individual) and Tween 80 and Tween 85 (concurrent) to allow for a greater number of scans for Span 80, which would otherwise be limited by the long scan times of the precursor ion scan used to quantify the Tweens. Also, the mass range of the precursor ion scan used to quantify Tween 80 and Tween 85 was truncated to focus on the oligomers of greatest abundance in these complex mixtures (m/z 640-930). This truncation was implemented to decrease the individual scan time of each precursor ion scan, increasing the total scan time for the high abundance oligomers, increasing sensitivity.

Calibration curves consisted of at least 5 standards and required a correlation coefficient of 0.99 or greater to be used for quantification. All calibration curves were 1/ $\times$ weighted, and standards whose calculated concentrations were beyond 20% of the intended concentration were removed from the calibration curve calculation. Calibration curves spanned from the lower limit of quantification (LLOQ) to the upper limit of quantification (ULOQ): for DOSS (0.2-25 $\mu\text{g L}^{-1}$), α -/ β -EHSS (0.2-23 $\mu\text{g L}^{-1}$), Span80 (60-300 $\mu\text{g L}^{-1}$), Tween 80 (60-300 $\mu\text{g L}^{-1}$), and Tween 85 (60-300 $\mu\text{g L}^{-1}$). Each calibration standard was spiked to give a final concentration of 500 ng L⁻¹ ¹³C₄-DOSS. Blank and check standards³³³3(3) were used for quality control purposes. Standards for DOSS fell within 20% of the spiked concentration and the non-ionic Corexit surfactants fell within 35% of the spiked concentration. All blank QC fell below the limit of detection.

A robust whole method error term was calculated as described by Gonzalez and Herrador, (2007), (2) using the following method: 250 ml of instant ocean IPA was spiked with standards to yield a concentration of 500 ng L⁻¹ DOSS and EHSS and

80,000 ng L⁻¹ non-ionics. This solution was divided between 50 ml aliquots and stored at -20°C until analysis. These aliquots were analyzed (5 ml, n = 4) on 4 different days to calculate an accuracy term that accounts for in day and between day variability. On each day of analysis, standards (all analytes) and internal standards (ionics only) were made fresh and used to calibrate the instrument. The whole method error was: DOSS (±7.3%), Tween 80 (±24.0%), Tween 85 (±23.3%), and Span 80 (±76%).

A4.3 GC-FID and GC-MS for Alkane Analysis. For alkane analysis by GC-FID, Agilent 5890 GC-FID system was used and the injection of 1 µL made with the injector at 300°C in splitless mode. The flow of the ultrahigh purity (UHP) Helium through a 100% dimethylpolysiloxane GC column (DB-1HT, 30 m × 0.25 mm ID × 0.1 µm thick film) was set at the constant pressure of 120 kPa. The GC was operated in a temperature program mode with an initial column temperature of 50°C for 1.4 min then increased to 350°C at a rate of 20°C/min and hold for 3 min.² The concentration of alkanes were determined from external calibration standards with a calibration range of 0.02 to 42.40 ng/µL.

For alkane analysis by GC-MS, Agilent 6890 GC-MS system was used equipped with an Agilent 5973 mass selective detector (MSD) and fitted with a 5% diphenyl/95% dimethyl polysiloxane capillary column (HP-5msUI, 30 m × 0.25 mm ID × 0.25 µm thick film). The injection of 1 µL made with the injector at 300°C in splitless mode. The flow of the UHP Helium through the GC column was set at the linear velocity of 36 cm s⁻¹. The oven program was at 50°C for 5 min, ramp at 10°C/min until 300°C, then hold for 24 min. Methyl decanoate, Methyl arachidate and Methyl octacosanoate were used as internal standards for quantification. Quantitation ions for detection of alkanes were at a *m/z* of 57 and 85 and the internal standard compounds at a *m/z* of 74 and 87.² Standards for calibration were used in each batch of experimental samples to control the performance of the GC-MS/GC-FID analysis.

Table A4.1. Average ejection rate ($\mu\text{g min}^{-1}$) for VOCs (C10-C14), IVOCs (C15-C19), SVOCs (C20-C24) and NVOCs (C25-C29) collected by nozzle and ESP. Ejection rates are reported for reactor injection rates of 20 and 50 $\mu\text{L min}^{-1}$ as the mean of duplicate data points.

	Injection Rate $\mu\text{L min}^{-1}$	Nozzle				ESP			
		C10-C14 $\mu\text{g min}^{-1}$	C15-C19 $\mu\text{g min}^{-1}$	C20-C24 $\mu\text{g min}^{-1}$	C25-C29 $\mu\text{g min}^{-1}$	C10-C14 $\mu\text{g min}^{-1}$	C15-C19 $\mu\text{g min}^{-1}$	C20-C24 $\mu\text{g min}^{-1}$	C25-C29 $\mu\text{g min}^{-1}$
Oil (NaCl)	Blank	NC	NC	NC	NC	NC	NC	NC	NC
	20	190	7.7	0.28	0.28	NC	0.07	0.18	0.10
	50	340	8.3	0.40	0.35	NC	0.09	0.19	0.11
Oil (Seawater)	Blank	NC	NC	NC	NC	NC	NC	NC	NC
	20	210	8.1	0.47	0.28	NC	0.10	0.17	0.14
	50	340	8.3	0.67	0.42	NC	0.19	0.33	0.22
Corexit 9527A (NaCl)	Blank	NC	NC	NC	NC	NC	NC	NC	NC
	20	170	9.2	0.48	0.40	NC	0.33	0.30	0.25
	50	280	11	0.83	0.63	NC	0.67	0.40	0.30
Corexit 9500A (NaCl)	Blank	NC	NC	NC	NC	NC	NC	NC	NC
	20	200	11	0.59	0.46	NC	0.23	0.36	0.20
	50	330	14	0.96	0.56	NC	0.54	0.39	0.27
Corexit 9500A (Seawater)	Blank	NC	NC	0.12	0.76	NC	NC	NC	NC
	20	190	10	0.77	1.2	NC	0.23	0.30	0.25
	50	360	11	1.1	1.5	NC	0.34	0.40	0.36

NC=Not calculated [†]

[†] The average ejection rates for blank samples and VOCs (C10-C14) in ESP collection system were not calculated as the concentration of alkanes were below LOD.

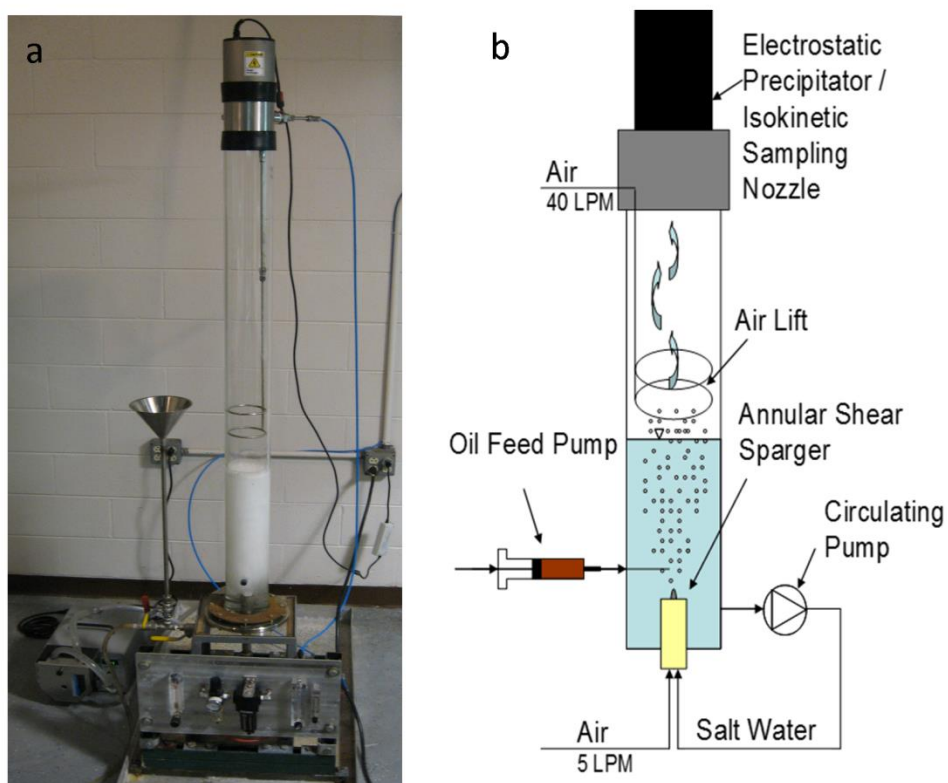


Figure A4.1: (a) The actual picture of the bubble column reactor. (b) The schematic of the bubble column reactor adapted from Smith et al.¹ and reprinted from Ehrenhauser et al.²

A4.2 Literature Cited.

1. J. S. Smith; L. F. Burns; K. T. Valsaraj; Thibodeaux, L. J., Bubble Column Reactors for Wastewater Treatment. 2. The Effect of Sparger Design on Sublation Column Hydrodynamics in the Homogeneous Flow Regime. *Industrial and Engineering Chemistry Research* **1996**, 35, (5), 1700-1710.
2. F. S. Ehrenhauser; P. Avij; X. Shu; V. Dugas; I. Woodson; T. Liyana-Arachchi; Z. Zhang; F. R. Hung; Valsaraj, K. T., Bubble bursting as an aerosol generation mechanism during an oil spill in the deep-sea environment : Laboratory experimental demonstration of the transport pathway. *Environ. Sci.: Processes Impacts* **2014**, 16, (1), 65-73.
3. B. J. Place; M. J. Perkins; E. Sinclair; A. L. Barsamian; P. R. Blakemore; Field, J. A., Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. *Deep-Sea Research* **2014**.