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Bioconcentration of Corexit® Dispersant Surfactant in the Oyster *Crassostrea gigas*

Andrea Boorse[1]* and Catherine Molloseau[2]
Student[1], Teacher[2]: Oregon Episcopal School, 6300 SW Nicol Road, Portland,OR 97223
*Correspondence: boorsea@go.oes.edu

Abstract

The oil dispersants Corexit 9527 and 9500 A were used in mass quantities in treatment of the Deep Water Horizon Gulf of Mexico oil spill of 2010. There has been much concern and controversy over the effect and toxicity of these oil dispersants on marine biota. Studies were conducted by EPA in 2010 on the toxicity of various oil dispersants and their oil mixtures. Primary active ingredients in oil dispersants are surfactants. To date, very little research has been conducted on the effect, toxicity or bioaccumulation of these surfactants on marine organisms. This investigation focused on the short term bioconcentration of Dioctyl Sulfosuccinate Sodium Salt (DOSS), the primary surfactant found in Corexit products, in the Pacific oyster *Crassostrea gigas*. DOSS is water soluble and fat soluble and has the potential to accumulate in the fatty tissues of organisms. Oysters were exposed to approximatley 500 µg/L of Corexit 9500 A over an approximate 60 hour period of time. Oyster and water samples were taken at approximately six hour intervals, frozen and shipped to the laboratory for DOSS analysis by liquid chromatography tandem mass spectroscopy (LC/MS/MS). The results of this bioconcentration study are discussed.

Introduction

This bioconcentration study of Dioctyl Sodium Sulfosuccinate (DOSS) in oysters, was conducted due to the extensive use of Corexit on the recent Deep Water Horizon oil spill in the Gulf of Mexico. Bioconcentration studies are important to determine if substances accumulate in organisms. It has been shown that DOSS is toxic to some marine organisms when they are directly exposed to high parts-per million (ppm) levels in water[1]. If DOSS is found to bioconcentrate, then marine organisms would build up toxic levels of the surfactant when exposed to very low concentration parts-per billion/parts-per trillion (ppb or ppt) levels which are typically found after dilution and turbulent mixing which occurs in the ocean[2].

Published studies concerning the bioconcentration of DOSS in marine biota were not found when the review of related research was conducted. Analytical methods for detecting DOSS in sea water and tissue are available and the methods are published. DOSS is an anionic surfactant used in Corexit 9500 A and 9527. Corexit 9500 A was the primary dispersant used in the 2010 Gulf oil spill. Corexit is sprayed over an oil slick and is used to break up the oil into smaller parts in order to speed up the process of eliminating the oil through evaporation and biodegradation. Corexit was also injected directly at the leak site to break up the oil before it reached the surface. The concentration of DOSS in the two Corexit products is about 30%[3]. Around 1.8 million gallons of Corexit were used in the gulf oil spill.

The question this study attempted to answer is whether DOSS will bioconcentrate in Pacific oysters. It was hypothesized that the DOSS will bioconcentrate in Pacific Oysters due to the fat soluble nature of the compound[4]. The bioconcentration exposure study was similar to the method described in the Journal of Experimental Marine Biology and Ecology[5]. The DOSS analysis was completed by Columbia Analytical Services using their standard operating procedure developed in 2010.

The study involved setup of a 20 gallon saltwater aquarium system with bottom filtration and circulation pump. An initial stability study for DOSS was completed in the aquarium water. The tank was then cleaned and setup again. Oysters were added to the tank and allowed acclimate. The tank was then spiked with Corexit 9500 A. Water samples and oysters were taken at various time intervals to be analyzed for DOSS levels. All samples were sent to the lab for LC/MS/MS analysis of DOSS concentrations. The concentration of DOSS in the tank water and in the oysters was calculated and plotted verses the exposure time.

Materials and Methods

A 20 gallon marine aquarium was set up with synthetic sea salt (Instant Ocean) and crushed coral filtration system oxygenated by an air pump circulation system. The density of salt water was set at approximately 1.020 g/mL. The temperature of the water ranged from 2 to 8 degrees C. An initial stability study for DOSS was completed by spiking the tank with 100 µLof Corexit 9500 A then sampling the water at various time intervals over a 75 hour period to determine the degradation rate of DOSS in the aquarium. The tank was then cleaned and setup again. Twenty oysters were added over several days. Once the oysters had acclimated to their environment, samples of water and oysters were taken as blanks. The tank was then spiked with 100 µL of Corexit 9500 A. Water samples and two oyster samples were taken at various time intervals (approximately six hours) to be analyzed for DOSS concentrations. Samples were stored in plastic bags, preserved at -18°C and sent to the lab for LC/MS/MS analysis of DOSS. Seawater samples were collected in 15 mL polypropylene centrifuge tubes. Samples were spiked with $^{13}C_4$ DOSS (Instrument Internal Standard) and $^{2}D_{34}$ DOSS (Surrogate) to monitor ionization suppression and extraction recovery, respectively. Seawater samples were extracted using Phenomenex Strata Solid Phase Extraction (SPE) cartridges. Oysters were shucked and the tissue frozen at -20*C then lyophilized (freeze dried). One gram of freeze dried oyster tissue was spiked with $^{13}C_4$ DOSS (Instrument Internal Standard) and $^{2}D_{34}$ DOSS (Surrogate) to monitor ionization suppression and extraction recovery, respectively. Freeze dried tissue samples were sonicated with 10 mL's methanol/dichloromethane solvent mix for three hours. A 2 mL subsample was taken to dryness and re-dissolved with 1 mL methanol. Liquid chromatography was performed using Shimadzu Prominence. Mass spectrometry was performed using ABSciex API 5000 operating in electrospray negative selective reaction monitoring (ESI – SRM) mode.

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Results

Table 1 shows data for DOSS in water samples taken from an initial stability study ran for DOSS in synthetic seawater with no oysters present. The data show a trend of decreasing concentration of DOSS in the water over the 75 hour period. This decrease may be due to biological degradation from by the added bacteria culture. This study shows that DOSS levels would remain at sufficient levels over the set period of time to complete the bioconcentration study. Figure 1 shows the decreases of the DOSS in the primary seawater study through the 75 hour time period. This study was done to determine if DOSS degraded in sea water prior to addition of the oysters.

Table 1. Primary Seawater Study

Trial	Time After Corexit was Added (hours)	DOSS in Seawater ug/L=ppb
1	1 hour	330
2	9 hours	330
3	21 hours	280
4	30 hours	290
5	75 hours	220

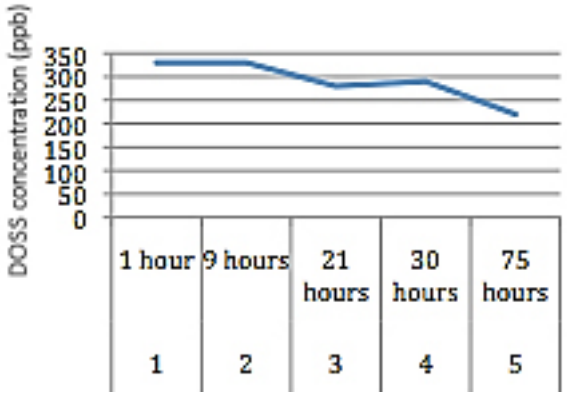

Figure 1. DOSS concentration over time for the primary seawater study

Table 2 shows the analytical results for DOSS concentrations in the oyster samples and water samples taken during the bioconcentration study. This data is also graphed in Figure 2. The data shows one anomaly, trial 2, with a DOSS concentration of 410 μ/kg, which is 60% higher than the overall average concentration of DOSS found in the oyster samples. This high value may be due to laboratory error. The data shows a parallel decrease in the DOSS concentration for oysters and water samples after 31 hours. This is assumed to be due to biodegradation of DOSS by the common soil bacteria Nitrsomonas/Nitrobacteria that was added to the tank. The bacteria culture was added to break down the toxic ammonia expelled by the oysters as waste. Slight degradation of DOSS was noted in the primary sea water study. This degradation did not affect the bioconcentration study.

Table 2. DOSS Data for Oyster/Seawater

Trial	Time After Corexit was Added	DOSS concentration in Oysters ug/kg	DOSS Sea Water ug/L
1	1 hour	290	210
2	7 hours	410	200
3	19 hours	270	150
4	25 hours	320	170
5	31 hours	250	210
6	43 hours	220	130
7	48 hours	180	130
8	55 hours	180	110
9	67 hours	180	94

Discussion

The results show that Dioctyl Sulfosuccinate Sodium Salt (DOSS) does not bioconcentrate in the Pacific oyster *Crassostrea gigas*. The data shows that the oyster equilibrated to the DOSS water concentration. Concentrations of DOSS declined in the oysters as concentration declined in the aquarium water. If bioconcentration had occurred the levels of DOSS would have steadily increased in the oysters as the exposure time to the DOSS increased. The Relative Percent Difference (RPD) in the analytical method is 30%. Error bars reflecting this RPD have been added to the graph of the data. All but one of the error bars for the oyster data and their respective aquarium water data overlap. Difference in matrices, sample prep and that the oysters contain approximately 10% solid may influence the oyster data thus shifting the overall DOSS concentration higher than the respective water sample data. The concentration of DOSS decreased in the oysters and water samples over time. It is believed that the decrease is due to biodegradation by the bacterial culture added to the aquarium water. It is unlikely that the oysters metabolized the DOSS being the decrease was noted in the primary seawater degradation study which did not involve oysters. There has been great concern in the Gulf of Mexico by the shellfish industry and government agencies about the effects trace levels of DOSS may have on shellfish. The results of this study are important in that it shows that DOSS does not bioconcentrate in oysters. Bioconcentration could have lead to the oyster's rapid accumulation of high levels of DOSS that could be toxic to the oyster or affect its physiology in some negative way. Further studies could test bioconcentration in other marine organisms and at higher water temperatures. Corexit crude oil mixes could be tested as well to see if bioconcentration is affected. Other studies could also involve environmental degradation rates of DOSS and DOSS oil mixtures. From my study it could be suggested that DOSS biodegrades relatively fast in the presence of bacteria. This degradation could be increased with temperature and exposure to sunlight as well.

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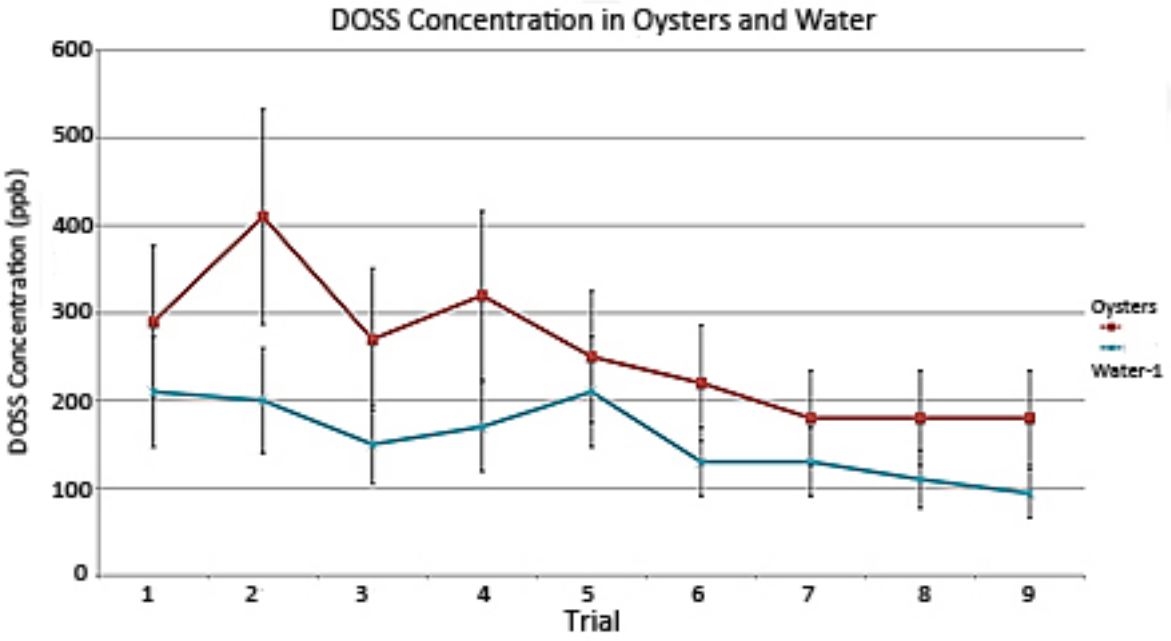

Figure 2. DOSS concentration over time for the oyster and salt water sampling study. The data show that there is no bioconcentration of DOSS occurring in the oysters.

References

1. Hemmer J. Michael, Barron G. Mace, and Greene M. Richard. (2010). *Comparative Toxicity of Eight Oil Dispersant Products on Two Gulf of Mexico Aquatic Test Species*. USEPA Office of Research and Development, pages 7-11.

2. George-Ares, A, & Clark, J.R. (2000). *Aquatic toxicity of two corexit dispersants.* Chemosphere. 40(8):897-906.

3. Scelfo, G.M.S, & Tjeerdema, *A simple method for determination of corexit 9527 in natural waters.* RST Marine Environmental Research, Volume 31, Issue 1, Pages 1-79 (1991).

4. Delphus, A.D. (2010, December 03). *Remarkably, in just 6 months NOAA and FDA scientists created a test for finding dispersant in gulf seafood.* Invention and Technology News.

5. Le Curieux-Belfond O., Fievet B., Seralini G.E., and Mathieu M. 2005. *Short-term bioaccumulation, circulation and metabolism of estradiol-17β in the oyster Crassostrea gigas.* Journal of Experimental Marine Biology and Ecology, 325 (2), pp. 125-133.