ACUTE TOXICITY OF COREXIT EC9500A AND ASSESSMENT OF DIOCTYL SULFOSUCCINATE AS AN INDICATOR FOR MONITORING FOUR OIL DISPERSANTS APPLIED TO DILUTED BITUMEN

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Environ Toxicol Chem., Accepted Article • DOI: 10.1002/etc.4065

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Corexit Toxicity and DOSS stability in dispersants

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This article contains online-only Supplemental Data

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Submitted 29 September 2017; Returned for Revision 12 November 2017; Accepted 20 December 2017

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Abstract: The current study investigated oil dispersant toxicity to fish species typical of the cooler regions of Canada, together with less well documented issues pertaining to oil dispersant monitoring. Corexit EC9500A oil dispersant toxicity was assessed for the fresh water fish species rainbow trout and seawater species coho, chinook, and chum, with a final LC50 acute lethality range between 35.3 mg/L – 59.8 mg/L. The LC50 range was calculated using confirmed 0 h dispersant concentrations that were justified by fish mortality within the first 24 h of exposure and by variability of the dispersant indicator dioctyl sulfosuccinate (DOSS) used to monitor concentration at later time-points. To investigate DOSS as an oil dispersant indicator in the environment, microcosm systems were prepared containing Corexit EC9500A, Finasol OSR52, Slickgone NS, and Slickgone EW dispersants together with diluted bitumen. The DOSS indicator recovery was found to be stable for up to 13 days at 5°C, but less than 8 days at ≥15°C. After 3 days at temperatures ≥15°C, the DOSS indicator recovery became unstable and was dependent upon multiple environmental factors including temperature, microbial activity, and aeration with potential for loss of solvents and stabilizers. A final assessment determined DOSS to be a discrepant indicator for long term monitoring of oil dispersant in seawater. This article is protected by copyright. All rights reserved

Keywords: Aquatic toxicology, Analytical chemistry, Analytical toxicology, Biodegradation, Corexit EC9500A, Environmental toxicology, Dioctyl sulfosuccinate (DOSS), Microcosm, Oil dispersant, Temperature
INTRODUCTION

Chemical dispersants are sometimes used as an oil spill countermeasure, enhancing the formation of microscopic oil droplets that move into the water column for dilution and dispersion (NRC, 2005). The aim of their application is to minimize surface contamination to coastal and surface species while promoting oil degradation (NRC, 2005). After an oil spill, currents and turbulence can cause mechanical oil droplet formation allowing smaller droplets to move into the water column (Yapa, 2012; NRC, 2005). Dispersants help stimulate this process by reducing the surface tension of the oil slick through amphipathic surfactant interaction at the surface of the oil droplet (Mearns, 2001). Biodegradation of the droplets occurs at the hydrocarbon-water interface, thus increasing the surface area of the droplets can have a considerable impact on the degradation and removal of spilled oil from the environment. Previous studies have established that under marine conditions chemically dispersed oil droplets are neutrally buoyant, following current patterns (Mearns, 2001; NRC, 2005). A number of publications support the view that dispersants stimulate oil biodegradation (Prince, 2013; Brakstad, 2015; North, 2015), while other researchers reported a negative effect on microbial hydrocarbon degradation rates (Kleindienst, 2015).

Future oil transport proposals in the Pacific North West have created particular interest in determining dispersant toxicities, as well as developing methods for monitoring oil dispersants environmental persistence. Transportation of petroleum products from the Canadian oil sands region has raised concerns regarding the potential for a diluted bitumen (dilbit) spill and the remediation techniques used to remove dilbit from the environment. Bitumen based oil is currently transported through pipelines and by tankers in the form of dilbit (bitumen with organic
solvents), with potential for substantial environmental impacts in the case of a leakage or spill (Winter, 2014). Dilbit export in the British Columbia (BC) region is anticipated to increase coastal traffic, making it essential to study effective oil spill response techniques, including toxicity testing and monitoring of dispersants that are appropriate to the sensitive Pacific coastal region of Western Canada.

Although generally considered to reduce harmful environmental impacts on the ocean’s surface, dispersant application may adversely affect species in the water column, such as fish populations (NRC, 2005). Toxicology data has also shown that dispersants may increase the biological uptake of toxic materials sourced from crude oil, such as polycyclic aromatic hydrocarbons (PAHs), resulting in an elevated aquatic toxicity in certain species (Singer, 2000; Ramachandran, 2004; Greer, 2012; Campo, 2013; Almeda, 2014). Due to high profile oil spills (i.e., DeepWater Horizon, Exxon Valdez), most dispersant research has focused on the toxicity of specific oil types and Corexit EC9500A CEWAFs (chemically enhanced water accommodated fractions), with a paucity of literature establishing dispersant LC50 toxicity ranges for north Pacific salmonoid and trout species. Historically, dispersants have had various toxicity classifications ranging from highly toxic to practically non-toxic (EPA toxicity categories), raising concerns over their application to sensitive ocean environments (Singer, 2000; Ramachandran, 2004; Greer, 2012; Almeda, 2014). Second and third generation dispersants, including Corexit EC9500A, have reported LC50 concentrations ranging from 25.2 mg/L to 130 mg/L for temperate *M. beryllina* (Hemmer, 2011), while some studies have supported a practically nontoxic LC50 concentration above 200 mg/L (Hemmer, 2011; Fuller, 2001; Fingas, 2002). Other research has also shown the potential for environmental factors such as temperature and salinity to affect Corexit EC9500A toxicity and chemical behaviour (Gardiner, 2013;
George-Ares, 2000). The wide range of LC50 concentrations and susceptibility to environmental influences emphasizes the need for a standardized test to determine regional and species specific effects on Corexit EC9500A toxicity.

As a consequence of toxicity concerns, the monitoring of oil spill dispersants post application has also become an environmental priority (Ramirez, 2013; White, 2014). The most common indicator for monitoring a number of oil spill dispersants is dioctyl sulfosuccinate (DOSS) (Kujawinski, 2011; Campo, 2013; Ramirez, 2013). DOSS is an anionic surfactant commonly associated to Corexit EC9500A, but is also present in other dispersants, such as Finasol OSR52, Slickgone NS and Slickgone EW (unpublished data). The monitoring of DOSS concentration levels in environmental seawater samples (after Corexit EC9500A application) has shown variable results with studies reporting both short and long term persistence of the surfactant (Kujawinski, 2011; Gray, 2013; White, 2014). Some studies have supported the potential for DOSS to undergo abiotic hydrolysis to monoctyl sulfosuccinate (MOSS) when stimulated by intense irradiation and bacterial degradation in seawater (Batchu, 2014; Campo, 2013). Others, however, have reported the presence of DOSS in the environment for up to 4 years after dispersant application and hypothesized that DOSS becomes protected by associating with bulk organic matter and minerals (White, 2014). In the event of a spill, the current standard analysis method uses DOSS as an indicator making it important to determine the stability of DOSS in a marine environment and the various factors that can affect the reported results.

Three main objectives of the current work were to: (i) determine the acute lethality of Corexit EC9500A in seawater water to fish species relevant to the cooler Pacific north-west, (ii) assess the effect of environmental factors on dispersant monitoring in the presence and absence of dilbit, and (iii) identify sampling conditions and constraints of selecting DOSS as an indicator.
for dispersant concentration. To achieve these objectives, a series of experiments were designed and carried out. The toxicity study included a series of Corexit EC9500A 96 h LC50 (50% lethal concentration) bioassays where the DOSS component was used to infer dispersant concentrations. Studies were initiated based on a previously reported 96h LC50 toxicity for rainbow trout (Oncorhynchus mykiss) of 354 mg/L to Corexit EC9500A in fresh water (ExxonMobil, 2008; Environment Canada, 2017), but aimed to confirm toxicity under marine conditions relevant to a single Corexit EC9500A application in the Pacific north-west. Further marine microcosm studies were carried out to identify environmental factors affecting the DOSS indicator’s persistence including salinity, UV, temperature, and aeration. The four commercially available oil dispersant blends selected were Corexit EC9500A, Finasol OSR52, Slickgone NS and Slickgone EW. Note that currently Corexit EC9500A is the only approved dispersant in Canada for open ocean marine dilbit spills. Additional experiments investigated potential microbial biodegradation, the effect of cations, and evaporation of volatiles by agitation through gas bubbling. The current results were used to assess the application of DOSS as a monitoring indicator for oil dispersants in response to a potential dilbit oil spill in Canadian north-west coastal waters.

MATERIALS AND METHODS

Reagents and supplies

Freshwater was collected from a well at the Environment and Climate Change Canada (ECCC) laboratory site in North Vancouver, BC, and with a hardness of 96.7 mg/L CaCO₃ and a pH of 7.85 ±0.10.

A fresh supply of seawater for the LC50 and microcosm studies was provided from an on-site pumping system and sand-filtered from the Burrard Inlet, BC, at a depth of 33 metres.
The seawater had a salinity range of 26 ppt to 32 ppt, hardness of 5290 mg/L to 6030 mg/L CaCO₃, and pH values of 7.75±0.10 to 7.97±0.10.

A sample of dilbit from the Cold Lake region was acquired from the Emergencies Science and Technology Section of Environment and Climate Change Canada (ESTS) in 3.78 L metal cans. Cold Lake Blend is a mixture of naturally occurring bitumen (high molecular weight hydrocarbons) blended with a light diluent to attain properties suitable for transport by pipeline. The dilbit sample is “sour” with approximately 4% sulphur content, and composed of approximately 70-80% bitumen and 20-30% diluent (Government of Canada, 2013; Yang, 2014).

Formic Acid (98% purity), acetic acid, DOSS 98% purity, and internal standard sodium bis(2ethylhexyl-d17) sulfosuccinate (DOSS-d34) 98 atom % deuterated were purchased from Sigma Aldrich (Oakville, ON, Canada). HPLC grade acetonitrile and iso-propyl alcohol were obtained from EMD Chemicals Inc. (Toronto, ON, Canada). Ultra-high purity (UHP) water at \( \geq 18.2 \text{ M} \Omega \text{cm}^{-1} \) was supplied by a Millipore MilliQ Plus system.

Dispersants employed, namely Corexit EC9500A, Finasol OSR52, Slickgone NS, and Slickgone EW, were supplied to ECCC by the Canadian Coast Guard and the Centre for Offshore Oil, Gas and Energy Research (COOGER), Fisheries and Oceans Canada.

Glassware used throughout this experiment was cleaned with detergent that did not contain DOSS. Following cleaning, the glassware was rinsed with solvent and purified water prior to heat treatment at 325 °C for over 12 h to remove trace organics.

Bioassay LC₅₀ experimental conditions

Toxicological 96 h LC₅₀ bioassays were conducted following standard ECCC reference testing methods for the freshwater species rainbow trout received from the Miracle Springs Trout
Hatchery in Mission, BC, and Rainbow Springs Hatchery, Ontario, Canada (Environment Canada, 1990). A single freshwater rainbow trout LC50 toxicity test was conducted in Moncton, New Brunswick (NB), using the trout from the Ontario hatchery while the second rainbow trout test was conducted in North Vancouver, BC, using trout from the Mission, BC, hatchery. Both facilities implemented the same testing procedures as outlined in the ECCC reference testing methods with differences in water matrices further discussed in the Corexit EC9500A toxicity results section (Environment Canada, 1990). The marine water species, tested in North Vancouver, BC, were coho (Oncorhynchus kisutch), chinook (Oncohynchus tshawytscha), and chum salmon (Oncohynchus keta) fry received respectively from the Department of Fisheries and Oceans Chilliwack Hatchery, Capilano Hatchery, and Inch Creek Hatchery in BC, Canada. Average fish weights for each species were 1.62 g for rainbow trout from the Ontario Hatchery, 1.21 g for rainbow trout from the BC hatchery, 2.47 g for chum, 6.12 g for chinook, and 1.59 g for coho. The salmon species, originally in freshwater, were acclimatized to 28 - 31 ppt of salt water by increasing the salinity 5 ppt every 5 days. All fish were certified as disease free and acclimated to the test conditions of 15±1°C and aerated at 6.5±1 mL/min·L throughout the experiment (testing room conditions are elaborated upon in the Indoor microcosm experimental conditions section).

Corexit EC9500A dispersant was weighed and added to the 96 h LC50 bioassay tanks by dipping the weighing boats into the water repeatedly until all of the Corexit EC9500A had been dispersed (visually confirmed). The tanks were then stirred with a Teflon rod to ensure homogeneity prior to fish exposure. The nominal concentration of Corexit EC9500A ranged from 5 mg/L to 1000 mg/L. Ten organisms per concentration were exposed to the dispersed Corexit in separate tanks containing 25 – 40 L of water at fish loading density of 0.2 – 0.9 g/L.
with no feeding during the 96 hour testing period. Mortality and behavioural changes were observed after 0.33, 0.67, 1.33, 24, 48, 72 and 96 h of exposure to a standard static (nonrenewal) LC50 bioassay of Corexit EC9500A. Dead fish were removed from solutions when found. A phenol quality control reference toxicant test was conducted using fish from each hatchery to insure that organism sensitivity was within acceptable quality control warning chart limits. Water samples (10 mL) were collected by glass pipette from each solution at the beginning (0 h) and end (96 h) of the bioassay. Samples were immediately preserved as a solution containing 25% (v/v) acetonitrile and stored under dark refrigeration at 4 ±2°C. Final acute lethality LC50 concentrations were calculated with the analytically confirmed initial dispersant concentrations based on DOSS and an Untrimmed Spearman-Karber statistical analysis with 95% confidence limits using the CETIS™ Version 1.9.2 (Tidepool Scientific Software, McKinleyville, CA, USA) in accordance with ECCC regulations (Environment Canada, 1990). Statistical significance between species was also determined using an F-test following ECCC regulations.

Outdoor microcosm experimental conditions

Outdoor seawater microcosm systems were employed to compare the persistence of four commercially available oil spill dispersants (Corexit EC9500A, Finasol OSR52, Slickgone NS, and Slickgone EW) together with lightly weathered dilbit under conditions representative of the BC west coast. Dispersant concentration was monitored using DOSS as the single component indicator of all four dispersants with an interest in determining the suitability of this method to future water quality monitoring. Since many of the previously discussed mechanisms thought to influence DOSS stability occurs simultaneously during an oil spill (i.e., photodegradation, biodegradation, etc.), the outdoor microcosm experiment sought to attain a representative interpretation of the DOSS indicator’s environmental persistence. Seawater microcosms of 20L
covered glass tanks were set in a position to receive indirect sunlight in the morning and direct sunlight in the afternoon. These tests were conducted in North Vancouver, BC, (Coordinates: N49.308051, W-123.0014) in July and August of 2016. Over an 11 day experimental period the tanks experienced fluctuations in day time temperatures between 16.0°C and 26.5°C, with water temperatures ranging between 18.5°C and 23.1°C. The UVA exposure was recorded multiple times throughout each day (refer to Supplementary Information). Each tank contained seawater together with oil dispersant and lightly weathered dilbit. Control seawater and study tanks were stirred at a mixing speed of 380 rpm using a Teflon stir bar of 80 mm x 10 mm. The microcosms were under constant mixing without creating deep vortex, but with slight coning, as per the recommendations for standardized handling of petroleum products (Singer, 2000).

*Indoor microcosm experimental conditions*
While initially only outdoor microcosm experiments were envisaged to study the persistence of dispersants, the results prompted further study to investigate environmental factors affecting DOSS stability. To study the effects of temperature on the DOSS indicator, 20 L indoor microcosm tanks were stirred under the same conditions as that in the previous section, and were maintained in temperature regulated chambers having 16 h exposure to fluorescent light per day. The light was full spectrum (400 nm to 700 nm) fluorescent lighting of ~500 lux, 16 h light, 8 hr dark, with a 15 minute gradual light transition. The UVA exposure readings at 356 nm were 0 μW/cm² for all chambers where testing occurred and were assumed to reduce photosensitive reactions when compared to the outdoor microcosms. Corexit EC9500A, Finasol OSR52, Slickgone NS and Slickgone EW were tested in temperature controlled chambers set at 15±1°C. Two of the dispersants, Corexit EC9500A and Finasol OSR52, were selected as representatives for further study at 5°C, 10°C and 20°C (each ±1°C).

Dispersant and dilbit addition and sampling of microcosms

Due to the high viscosity of the dilbit, chemical dispersion did not occur readily and required a 1:10 (w/w) ratio of Corexit EC9500A to dilbit, despite previous studies recommending a 1:20 or 1:25 (w/w) ratio for crude oils (Madison, 2015). Ten mL of seawater was first vortexed with a 1:10 ratio of dispersant and dilbit in a glass centrifuge tube, and then poured directly into the microcosm tanks. This pre-mixing method improved the reproducibility of the results with no significant impact on DOSS recovery (unpublished results). The mixture was prepared to a final nominal concentration of 1 mg/L DOSS for each of the four dispersants in the microcosm tanks (refer to SI for SDS).

The study tanks were sampled daily for the first 4 days of the experimental period and as necessary afterwards. Five 7.5 mL CEWAF seawater samples were collected each day; three
were preserved with 2.5 mL of acetonitrile (as described above) immediately after collection and the other two samples had no acetonitrile added upon collection. All samples were stored in the same 4±2°C refrigerated room. For evaluating the stability test samples without acetonitrile, the samples were vortexed together with 2.5 mL of acetonitrile immediately after removal from the refrigerator and prior to analysis. Final recoveries of DOSS used the immediately preserved triplicate samples were averaged and the standard deviation used to determine confidence.

Procedure for study of microbial activity

A seawater control (non-sterile) and sterilized (autoclaved) seawater were employed as matrices for a microbial activity study. The seawater sterility was tested by adding 5 mL of autoclaved seawater into a test tube containing an inoculated, non-selective, non-differential bacterial growth medium Brain Heart Infusion Broth (BHIB). The sterility of the seawater was confirmed after incubating the sample for 48 h and observing no bacterial growth. This present study used stirred 2 L glass beakers maintained under aseptic conditions in a laminar flow hood at room temperature 21±2°C. The concentration of both DOSS and monooctyl sulfosuccinate (MOSS) in the seawater was monitored throughout the experiment by LC/MS/MS. Triplicate aliquots of 7.5 mL were taken from each study microcosm at 1, 3, 7, and 10 days after initiation. Samples were immediately preserved in a final v/v of 25% acetonitrile and store under dark refrigeration at 4 ±2°C.

Procedure for study of oxic/anoxic gas purging

Under the same experimental conditions specified in the Indoor microcosm experimental conditions section, an oxic and anoxic gas purging experiment was conducted using six 500 mL volumes of seawater and filtered seawater solutions of Corexit EC9500A in 600 mL glass beakers. The experiment used filtered seawater prepared with 0.45 µm Agilent PTFE filters to
remove any larger particulates in the seawater which DOSS could potentially adsorb onto (White, 2014). The seawater and filtered seawater systems either received no gas or were treated with bubbling 99.9% pure oxygen or 5.0 UHP nitrogen gas to monitor DOSS behaviour when exposed to aeration. A steady flow rate of 100±10 mL/min was maintained throughout the 168 h experimental period for both oxygen and nitrogen systems and temperature controlled at 15±1°C. Triplicate 3 mL sample aliquots were collected from all systems at 0 hr, 4 hr and 8 hr on the first day of testing and sampled as necessary (dependent on loss of DOSS rate) afterwards. Samples were immediately preserved in a final v/v of 25% acetonitrile and stored under dark refrigeration at 4 ±2°C.

Instrument analysis

A previously published direct injection method from this laboratory (Brunswick, 2015) was adapted and validated to determine DOSS concentrations in samples from the LC50 and microcosms experiments. The LC50 experiments had a quantitation range between 0.5 – 25 ppb. The microcosm experiments implemented a DOSS quantitation range of 10 – 200 ppb with lower injection volumes to reduce the dilutions needed for analysis (refer to SI for method validation). It is noted that the EPA benchmark for DOSS analysis in water samples is set at a 20 ppb Reporting Limit with a 40 ppb Aquatic Life Benchmark (U.S. E.P.A, 2013). Stock reference solutions were prepared by dissolving DOSS in a 90:10 % (v/v) solution of acetonitrile and UHP water. Final calibration standards and samples in 25:75 % (v/v) acetonitrile and seawater were analyzed using an Agilent Infinity 1290 HPLC with Infinity 1260 autosampler and Agilent 6490 iFunnel Series Liquid Chromatograph Tandem Mass Spectrometer (LC/MS/MS) controlled by MassHunter 6400 software. The chromatographic column used for this present study was a Poroshell 120 SB AQ column (2.7 µm, 2.1 mm x 50 mm, Agilent Technologies). Briefly, the
method employed electrospray negative mode ionization with ion transition for DOSS at 421.2 $m/z \rightarrow 80.9\ m/z$ with collision energy (CE) 25 eV and internal standard $D_{34}$DOSS at 455.4 $m/z \rightarrow 80.9\ m/z$ (CE 25 eV). Additionally where stated, the monooyctyl sulfosuccinate (MOSS) hydrolysis product of DOSS, was monitored by pseudo MRM at $309.1\ m/z \rightarrow 309.1\ m/z$ (CE 5 eV).

An LC/QTOF method was employed to confirm the precipitate samples. In this case, we used an Agilent 6550 Time of Flight Mass Spectrometer (LC/QTOF/MS) with Agilent 1290 Infinity autosampler, and Agilent 1290 HPLC and quaternary pump controlled by MassHunter 6400 software for qualitative determination of a DOSS precipitate collected in the oxic/anoxic gas purging experiment (discussed in the Oxic and anoxic gas purged systems section). The precipitate (from the experiment) and pure DOSS were dissolved in a 1:1:1 (v/v/v) mixture of UHP water, ACN and isopropanol (IPA) due to difficulties in solubilizing the extracted precipitate. The samples were then filtered using a sterile 0.45 μm PTFE filter to remove particulates.

RESULTS AND DISCUSSION

Corexit EC9500A toxicity results

Acute toxicity tests conducted on fresh water and seawater fish species established a 96h LC50 bioassay concentration range for Corexit EC9500A of 35.3 – 92.1 mg/L. The lethality of Corexit EC9500A dispersant occurred within the first 24 hours of assay. Generally, Corexit EC9500A exhibited a greater toxicity to the seawater species coho, chinook, and chum salmon with LC50 values ranging from 35.3 mg/L to 47.6 mg/L in comparison to the fresh water rainbow trout which ranged from 59.8 – 92.1 mg/L (Fig. 1). It was noted that the fresh water bioassay results significantly differed from a previously reported 96h LC50 value of 354 mg/L.
(rainbow trout) using the Corexit 9500 dispersant (Environment Canada, 1993), but concurred with more recently reported LC50s ranging from 25.2 mg/L to 130 mg/L (ExxonMobil, 2008; USEPA, 2010; Hemmer, 2010). Despite our efforts to obtain information from the manufacturer, some ambiguity exists regarding any differences in the formulation of Corexit EC9500A and that of Corexit 9500 from 1993. The rainbow trout also displayed variable acute toxicity dependent on the water source, regardless of matching water hardness. Studies using moderately hard (due to hardness matching) adjusted dechlorinated tap water (Moncton, NB, Canada) reported a 96h LC50 bioassay concentration of 92.1 mg/L, while Corexit EC9500A in fresh well water from North Vancouver, BC, Canada reported a lower LC50 of 59.8 mg/L (Fig. 1) (Environment Canada, 1990). For comparison, the well water conductivity was 410 µS/cm and the dechlorinated tap water was 323 µS/cm, respectively. Note that Corexit EC9500A is currently approved in Canada for open marine water use only, therefore fresh water studies are not as relevant when assessing its toxicity. The fresh well water Corexit EC9500A LC50 concentration value of 59.8 mg/L was determined as the final accepted toxicity for rainbow trout under currently specified local conditions. Further investigation of toxicity differences between fresh water and seawater was beyond the scope of the paper, but is recommended for future study.

During toxicity testing of Corexit EC9500A, the use of dioctyl sulfosuccinate (DOSS) as a monitoring indicator for all dispersant components was evaluated. Analysis of DOSS at 0h time points reflected the expected nominal concentrations of Corexit EC9500A (Table 1 and Table 2), while those at 96h time points were considerably more variable, particularly in the fresh well water and seawater systems. While the toxicity test conducted in Moncton, NB maintained recoveries of DOSS above 88%, the fresh well water source showed a loss in DOSS recovery of 32% for the nominal 10 mg/L Corexit EC9500A tank, while in the coho species
seawater studies DOSS recoveries ranged from <2% (below method detection limit) to 44%. The loss of DOSS was more significant for dispersant concentrations < 28 mg/L for seawater (Table 1) and <56 mg/L for fresh water (Table 2). DOSS represented only a single component between 5% and 25% of the dispersant blends (refer to MSDS) and the stability of the other components under current conditions is effectively unknown. While mean measured concentrations of the compound under study are typically employed for the 96h LC50 toxicity testing, in the current case, our work has shown that the DOSS concentration can be extremely unstable during the duration of the experiment and may not adequately assess the fate of all individual dispersant components. Due to this variability in the DOSS indicator and because all of the fish species under study died within 24h of experiment start, the authors felt justified in applying the nominal 0h confirmed dispersant concentration as a more accurate assessment of toxicity. Based on this assumption, the currently reported static (nonrenewal) results provide the best estimate of LC50 concentration for the dispersant under the experimental conditions. If alternatively the average DOSS concentration (i.e., 0 h and 96 h) was used in the calculation, the results would overestimate static (nonrenewal) application of Corexit EC9500A as more toxic than in actuality. For reference only, the averaged 0h and 96h LC50 concentrations are reported in Supplementary Information.

Microcosm study of four oils spill dispersants with dilbit

The outdoor seawater microcosms showed that the percent recovery of the DOSS indicator remained relatively stable over the first 3 days of exposure (Fig. 2). A minor observed increase was attributed to evaporation of water and petroleum distillate from the dispersant. Unexpectedly, during the 24h interval between the third and fourth day of testing, the percent recovery of DOSS dropped significantly for all four dispersants tested in this experiment. For
example, the average DOSS recovery from the Corexit EC9500A system reduced from 98% to 19% within the a 24 hour period between the third and fourth day, declining further to only 1% recovery by day 7 (Fig. 2). Similar trends were observed for the other dispersants, namely Finasol OSR52, Slickgone NS and Slickgone EW (Fig. 2). These results corresponded with the loss in DOSS previously described during the Corexit EC9500A 96h LC50 bioassay testing. The short time window that DOSS remains stable has many important implications on sample collection and oil dispersant analysis. The observed rapid decrease in DOSS after 3 days was, however, in disparity with published reports indicating the persistence of DOSS in a marine environment for up to 64 days (Kujawinski, 2011) and 4 years post dispersant application (White, 2014). As a result of the discrepancy, further microcosm studies were initiated under controlled laboratory conditions.

Dispersants in temperature regulated microcosms

The indoor seawater microcosms were conducted at temperatures representing the range of monthly surface water typical of the Pacific North West coast over the year 2016 (NOAA, accessed 2017). Results clearly showed a distinct temperature dependency in relation to DOSS recovery (Fig. 3 and Fig. 4). Warmer tank temperatures (15°C and 20°C) produced distinctly low ≤5% DOSS recovery after 5 to 8 days, while the colder temperatures (5°C and 10°C) maintained good DOSS recovery past the tenth day (Fig. 3 and Fig. 4). Since all other environmental factors were consistent in this microcosm study, we concluded that the DOSS component of the dispersants was subject to environmental losses at higher temperatures. The prolonged DOSS stability at colder temperatures is also much more consistent with samples collected from the Deepwater Horizon spill at depths of 1200 m (Kujawinski, 2014). Future
monitoring of oil dispersant impacted areas should carefully consider these temperature dependencies.

Other publications have highlighted UV and bacterial activity as likely instigators of DOSS degradation in aqueous solution (Campo, 2013; Batchu, 2014; Mobing, 2016). Photo-stimulated DOSS hydrolysis degradation reactions were previously reported as having a 14 day and 6.5 day half-life under constant exposure to UV light of 350 nm and simulated sun light (300 - 800 nm) respectively (Batchu, 2014). Both exposure conditions exhibited a much longer DOSS half-life than observed in the present study. The fluorescent lighting used in the indoor microcosm experiments emitted no detectable UVA light (tested at 356 nm) suggesting that the observed rapid loss of DOSS was unlikely to be directly caused by photodegradation as seen by Batchu (2014). The similar trends observed in the 20°C indoor microcosms and the outdoor microcosms (Fig. 2 and Fig. 3) also indicate that the much higher UVA exposure of the natural sunlight (Supplementary Information) had little effect on the DOSS indicator under these conditions. Conversely, bacterial activity could not be ruled out, although we observed no exponential decay of DOSS comparable to that reported by other authors using pre-conditioned bacteria in small flask systems (Campo, 2013). Meanwhile, a number of other potential mechanisms were identified that could also cause dispersant/surfactant removal from the environment, such as volatilisation across the sea/air interface, abiotic degradation (i.e., photolysis or hydrolysis), adsorption to particulates, association with cations, precipitation, and potential uptake by microorganisms and microbial degradation (Voss, 1963; McWilliams, 2000).

**Influences of cations on DOSS stability**

It was recognized that anionic surfactants such as DOSS are known to form aggregates in solution and the presence of cations increases the formation of salt bridges that stabilize densely
packed micelles (Sammalkorpi, 2009). To determine whether salts affected DOSS stability in
dispersants, Corexit EC9500A was added to microcosms of deionized water, water plus sodium
chloride, and water plus sodium chloride, calcium sulphate, and magnesium sulphate with cation
concentrations matched to BC coastal seawater. The results from deionized water with added
salts (Na⁺ or Na⁺ plus Ca²⁺ and Mg²⁺) indicated no loss of DOSS occurring over a 10 day period
at 20°C (refer to SI). By comparison, Corexit EC9500A applied to seawater had shown a DOSS
reduction to below 1% by day 7 (Supplementary Information). It was concluded that these
cations, or water hardness, were not principle factors affecting the stability of DOSS in the
seawater microcosms. However, the potential for cations to increase the toxicity of dispersants to
aquatic life still remains (Voss, 1963; Brunswick, 2015) and the current 96h LC50 bioassay
results for seawater support this rationale.

Influence of microbial activity

The cause of DOSS loss from the seawater microcosms and bioassay experiments was
investigated with respect to its potential microbial biodegradation. The persistence of DOSS
from Corexit EC9500A was monitored at 21±2°C in a seawater control (non-sterilized) versus
sterilized (autoclaved) seawater. The fresh seawater contained uncharacterized bacteria present
after pumping through a sand filtration system, while autoclaved seawater was confirmed to be
sterile (see the Procedure for study of microbial activity section). An initial experiment using
sealed containers to minimize airborne bacterial contamination resulted in prolonged DOSS
stability in both sterilized and unsterilized conditions (unpublished result). The alternative use of
a laminar flow hood, to maintain aseptic conditions together with open containers, allowed for
evaporation of volatile petroleum distillates from the Corexit EC9500A dispersant as a
comparable to in situ environmental exposure. Under these conditions, for both the sterile and
non-sterile seawater systems, the DOSS component of Corexit EC9500A remained consistent over the first 3 days (Fig. 5). By the seventh day of the study, DOSS recovery dropped significantly in the non-sterile solution (Fig. 5). In contrast, the sterile seawater matrix showed no comparable loss of DOSS from the system (Fig. 5). Results indicated that microbial activity was involved in the rapid disappearance of DOSS from the seawater matrix, which was in line with data from a low volume microcosm study using preconditioned bacteria by Campo (2013). It is predicted that DOSS disappearance could occur either directly by microbial uptake and biodegradation, or indirectly by induced abiotic degradation, precipitation, adhering to particulates, and chemical hydrolysis.

Oxic and anoxic gas purged systems

While the presence of microbial activity was a contributing factor to the loss of DOSS from dispersant treated seawater microcosm systems, one could not rule out that other additional factors were affecting the stability of DOSS. The airflow required in the microbial activity experiment led to speculations that evaporation of the more volatile components from the oil dispersant may contribute to the destabilization of DOSS. Additional experiments were conducted where control seawater and filtered seawater systems were maintained at 15±1 °C, and arranged alongside of equivalent systems vigorously bubbled at a flow rate of 100 mL/min with either 99.993% pure oxygen (O₂) or 99.99% ultra-high purity nitrogen (N₂) for the entire period of the experiment. Systems were sampled three times on the first day, and as necessary until the end of the experiment (168 h). Both the seawater and filtered seawater systems (containing Corexit EC9500A) experienced a significantly faster loss of DOSS than systems with no gas purging treatments (Fig. 6). There is a general consensus that anaerobic biodegradation of oil dispersants is typically a slower process than under aerobic conditions (Das, 2011; McGenity,
2012; Campo, 2013). Thus if DOSS loss was entirely dependent upon aerobic microbial biodegradation, it would be expected that the \( \text{N}_2 \) purged system would result in significantly less DOSS loss in comparison with the \( \text{O}_2 \) system. As this was not the case, the study result indicated that there were additional abiotic mechanisms inducing loss of DOSS.

In the final stages of the experiment, an unknown white precipitate formed on the end of all the capillaries which were bubbling \( \text{N}_2 \) or \( \text{O}_2 \) gas into the seawater systems. Further investigation of this compound using the LC-QToF determined that DOSS was present, suggesting that the accelerated loss of the more volatile components may have encouraged some DOSS precipitation (Fig. 7). Previously, pure DOSS had been easily dissolved in a 90\% v/v acetonitrile and UHP water mixture to prepare the stock solutions (Materials and Methods). However, the unknown white precipitate demonstrated poor solubility in acetonitrile and water mixtures with dissolution occurring only in the presence of isopropanol (unpublished result). Low DOSS solubility was speculated to be caused by Corexit EC9500A or seawater chemical species associated with the precipitate such as glycols or divalent salts (common white precipitates in seawater). The seawater and filtered seawater control systems (no gas) contained no visible traces of a white precipitate attributing precipitate formation to the increased agitation from the gas bubbling. Due to relatively low concentrations (1 \( \mu \text{g/mL} \) of DOSS), it is proposed that the capillary tip may have encouraged nucleation after the solvents had evaporated, allowing for visual confirmation of the precipitate.

Our results demonstrated that loss of dispersant DOSS in seawater was potentially not just due to microbial activity, but was additionally affected by other abiotic factors that included evaporation of petroleum distillates and potential precipitation of DOSS. In fact, the oil spill dispersants contain a significant percentage (see SI for composition details) of volatile
hydrocarbons which, upon evaporation, could potentially lead to DOSS instability in seawater. Below the surface in larger bodies of water, lower temperatures slow biodegradation and chemical reactions, but the removal of solvating agents may increase the potential for DOSS to precipitate and adhere to micro-particulates. This current data is also consistent with previous publications reporting the marine persistence of DOSS from dispersant application for up to 64 days at a depth of 1000 to 1200 m (Kujawinski, 2011) and its presence in sand, sediment, and coral for up to 4 years (White, 2014). It would be expected that very little evaporation or degradation of the oil dispersant solvents would occur when applied at depths of a kilometer below the surface, resulting in the prolonged stability of DOSS in the water column. As well, the presence of DOSS in sand, sediment, and coral for up to 4 years could be a consequence of the eventual evaporation or degradation of the dispersant solvents with subsequent DOSS deposition onto the sea floor. A further observation of this present study showed little to no significant differences between the seawater and filtered seawater suggesting that particulates >0.45 μm do not play a large role in initial DOSS destabilization.

CONCLUSIONS

The main aim of the current study was to assess the acute lethality of Corexit EC9500A and to monitor oil dispersants after a potential dilbit oil spill in the Canadian North-West coastal waters. The observed toxicity of Corexit EC9500A oil spill dispersant ranged from 59.8 mg/L to 92.1 mg/L for fresh water rainbow trout and 35.3 mg/L to 47.6 mg/L for seawater chinook, chum, and coho salmon species. The use of confirmed 0 h dispersant concentrations in the toxicity calculation were justified by a number of factors, namely the observed instability of the DOSS indicator compound, the diverse compound blend of the dispersants, and fish lethality occurring within the first twenty four hours of assay.

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The secondary objective of the present study was to gain insights on the persistence of four commercially available dispersants, namely Corexit EC9500A, Finasol OSR 52, Slickgone NS and Slickgone EW in a northern marine environment. The DOSS indicator component used to monitor for the dispersants was found to be temperature dependent. DOSS, an accepted chemical indicator for dispersant monitoring, persisted longer under colder temperatures (5°C and 10°C, 10 days) compared with warmer temperatures (15°C and 20°C, 5 to 8 days). It was concluded that DOSS could be used as an indicator for dispersant concentration for up to 3 days in a seawater environment <20°C. After 3 days, DOSS stability became dependent upon biotic and abiotic environmental factors.

The third aim of this present study focused on determining the cause of DOSS instability that was observed in seawater microcosms. Data collected under sterile and non-sterile seawater conditions confirmed microbial activity was significant in DOSS loss, while further experimentation revealed that other abiotic mechanisms were also involved. While the seawater and fresh water matrices clearly showed a more definitive loss of DOSS than the Moncton, NB, city tap water, an assessment of sodium, calcium, and magnesium components (ions that differed between the two water types) demonstrated that these cationic salts did not stimulate the process. It was concluded that while water type appeared to play a role in dispersant toxicity, it did not affect the loss of DOSS in a seawater matrix. The presented N₂ and O₂ gas bubbling experiment suggested that evaporation of volatile dispersant solvents and stabilizers contributed to inducing DOSS precipitation in both seawater and filtered seawater solutions. The dispersant solvent evaporation rate has the potential to affect both the stability of DOSS as an indicator to monitor oil dispersant in the water column, as well as the potential for DOSS deposition on the seabed. In
relating to DOSS as a dispersant indicator, its concentration was concluded to be potentially non-representative of dispersant components as a consequence of DOSS variability over time.

**List of Abbreviations**

- CEWAF = Chemically Enhanced Water Accommodated Fraction
- Dilbit = diluted bitumen
- DOSS = dioctyl sulfosuccinate, C_{20}H_{37}O_{7}S
- ECCC = Environment and Climate Change Canada
- LC50 = lethal concentration to 50% of test organisms
- LC/MS/MS = liquid chromatography triple quadrupole mass spectrometry
- MDL = method detection limit
- MOSS = monoctyl sulfosuccinate, C_{12}H_{21}O_{7}S
- MRM = multiple reaction monitoring
- SDS = Safety Data Sheet

**Supplemental Data**—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

**Acknowledgment**—The authors would like to acknowledge A. Huybers, P. Jackman and the Environmental Toxicology team of the Atlantic Laboratory for Environmental Testing of Environment & Climate Change Canada for their support and discussion. We would also like to thank P. Thompson, M. Menacherry, and T. Wang (Marine Water Quality Monitoring, Pacific Environmental Science Centre of Environment & Climate Change Canada) for the valuable discussions and insights. Much appreciation is also extended to our colleagues H. Kwok, L. Chow and O. Blajkevitch (Organic Chemistry, Pacific & Yukon Laboratory for Environmental Testing of Environment & Climate Change Canada) and C. Le (SFU Co-op student,
Environmental Toxicology, Pacific & Yukon Laboratory for Environmental Testing of Environment & Climate Change Canada) for their valuable assistance and involvement. Both C. MacInnis and G. Park would like to acknowledge the UBC Science Co-op program for their continued support in the process.

Data availability—Data, associated metadata, and calculation tools are available from the corresponding authors (dayue.shang@canada.ca, graham.vanaggelen@canada.ca).
REFERENCES


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Figure 1. Summary of 96h LC50 bioassay concentrations for fresh and seawater fish species using confirmed initial 0 h Corexit EC9500A concentrations with 95% confidence intervals.

Figure 2. DOSS percent recovery from Corexit EC9500A, Finasol OSR52, Slickgone NS, and Slickgone EW microcosms exposed to outdoor conditions.

Figure 3. Recovery of DOSS from Corexit EC9500A treated microcosm systems under temperature controlled conditions.

Figure 4. Recovery of DOSS from Finasol OSR52 treated microcosm systems under temperature controlled conditions.

Figure 5. Recovery of DOSS from Corexit EC9500A treated sterile and non-sterile seawater solutions.

Figure 6. Recovery of DOSS from Corexit EC9500A treated seawater and filtered seawater solutions under aerobic and anaerobic conditions.

Figure 7. Example chromatographs comparing the solvent blank (black), pure DOSS (red), and the DOSS precipitate (blue) collected from the aerobic gas purged systems analyzed in negative mode on the LC-QTOF for 421.2265 m/z.
Table 1. Concentration of Corexit EC9500A in Seawater Toxicity Tests

<table>
<thead>
<tr>
<th>Nominal Conc. (mg/L)</th>
<th>Chum</th>
<th>Coho</th>
<th>Chinook</th>
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<tr>
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<tr>
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Note: Confirmatory concentrations of Corexit EC9500A in seawater for Chum (N=1), Coho (N=2) and Chinook (N=1) toxicity study.

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Table 2. Concentration of Corexit EC 9500A in Freshwater Toxicity Tests

<table>
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$^a$Confirmatory concentrations of Corexit EC9500A in freshwater for rainbow (R.) trout toxicity tests

$^b$Conducted in Moncton, New Brunswick (N=2)

$^c$Conducted in North Vancouver, BC (N=2)
**Summary of 96h LC50 bioassay concentrations for fresh and seawater fish species using confirmed initial 0 h Corexit EC9500A concentrations with 95% confidence intervals**

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