

Comparative Toxicity of Two Chemical Dispersants and Dispersed Oil in Estuarine Organisms

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Abstract

Chemical dispersants can be a useful tool to mitigate oil spills. This study examined potential risks to sensitive estuarine species by comparing the toxicity of two dispersants (Corexit[®] EC9500A and Finasol[®] OSR 52) individually and in chemically enhanced water-accommodated fractions (CEWAFs) of Louisiana Sweet Crude oil. Acute toxicity thresholds and sublethal biomarker responses

were determined in seven species (sheepshead minnow, grass shrimp, mysid, amphipod, polychaete, hard clam, mud snail). Comparing median lethal (LC_{50}) values for the dispersants, Finasol was generally more toxic than Corexit and had greater sublethal toxicity (impaired embryonic hatching, increased lipid peroxidation, decreased acetylcholinesterase activity). The nominal concentration-based mean LC_{50} for all species tested with Corexit was 150.31 mg/L compared with 43.27 mg/L with Finasol. Comparing the toxicity of the CEWAFs using the nominal concentrations (% CEWAF), Corexit-CEWAFs appeared more toxic than Finasol-CEWAFs; however, when LC_{50} values were calculated using measured hydrocarbon concentrations, the Finasol-CEWAFs were more toxic. There was greater dispersion efficiency leading to greater hydrocarbon concentrations measured in the Corexit-CEWAF solutions than in equivalent Finasol-CEWAF solutions. The measured concentration-based mean LC_{50} values for all species tested with Corexit-CEWAF were 261.96 mg/L total extractable hydrocarbons (TEH) and 2.95 mg/L total polycyclic aromatic hydrocarbons (PAH), whereas the mean LC_{50} values for all species tested with Finasol-CEWAF were 23.19 mg/L TEH and 0.49 mg/L total PAH. Larval life stages were generally more sensitive to dispersants and dispersed oil than adult life stages within a species. These results will help to inform management decisions regarding the use of oil-spill dispersants.

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Electronic supplementary material

The online version of this article (doi:10.1007/s00244-017-0430-9) contains supplementary material, which is available to authorized users.

Following an oil spill, dispersants are applied to alter the chemical composition of the oil by decreasing interfacial tension and breaking up the oil into particulate-sized droplets (Council 2005). Smaller droplets of oil contain a higher surface area, allowing bacteria to degrade the oil more quickly. The use of dispersants may reduce the overall impact of an oil spill (Lessard and Demarco 2000); however, dispersing oil into water may result in increased chemical loading into benthic and coastal habitats (Ramachandran et al. 2004). Current and tidal movement may transport dispersants into sensitive coastal habitats such as mangroves and salt marshes. Dispersed oil droplets may become trapped and concentrate in semi-enclosed coastal areas (Scarlett et al. 2005).

The 2010 Deepwater Horizon Oil Spill was treated with approximately 7 million liters of the dispersant Corexit[®] 9500A. Finasol[®] OSR 52 is another dispersant registered for oil spill response in the United States, but considerably less is known regarding its toxicity to estuarine species. Corexit is manufactured by Nalco Energy Services, Sugar Land, TX, and Finasol is produced by Total Fluides, Paris-La Defense, France. Both dispersant compounds consist of mixtures of petroleum distillates and surfactants, but the exact chemical make-up differs. The list of chemicals in Corexit is publically available (<https://archive.epa.gov/bpspill/web/html/dispersants-qanda.html#list>), whereas the constituents of Finasol remain undisclosed to the public.

The objectives of this study were (1) to compare the acute toxicity of two oil spill dispersants, Corexit and Finasol, in a suite of common estuarine species and (2) to compare the acute toxicity of dispersed oil preparations [chemically enhanced water accommodated fractions (CEWAFs) of each dispersant with Louisiana Sweet Crude (LSC) oil] in a suite of common estuarine species.

The species chosen for study are common to southeastern tidal creek estuaries and represent different habitats and trophic levels within the ecosystem. The test organisms included a fish (sheepshead minnow, *Cyprinodon variegatus*), crustaceans (grass shrimp, *Palaemonetes pugio* and mysid, *Americamysis bahia*), a gastropod (Eastern mud snail, *Ilyanassa obsoleta*), a bivalve mollusc (hard clam, *Mercenaria mercenaria*), a polychaete (nereid worm, *Neanthes arenaceodentata*), and an amphipod (malacostracan, *Leptocheirus plumulosus*). These seven estuarine species are of ecological and economic importance, contributing important functions, such as influencing phytoplankton and nutrient dynamics, serving as prey for commercially and recreationally important fish species, and providing a source of commercial shellfish revenue (hard clam). For fish, shrimp, clams, and snails, the sensitivity of more than one life stage was assessed. Median lethal (LC₅₀) toxicity values were determined for each test organism and a number of sublethal endpoints were measured in some species, including timing and success of embryo hatching, p450 enzyme activity, acetylcholinesterase activity, splenocyte proliferation, and lipid peroxidation activity. The results of this study may aid resource managers' capacity to respond to oil spills by increasing scientific knowledge of the impacts of oil, with and without chemical dispersants, on estuarine salt marsh ecosystems.

Materials and Methods

Test Species and Conditions

C. variegatus embryos and adults and juvenile *L. plumulosus* and *A. bahia* were acquired from Aquatic Biosystems (Fort Collins, CO). Adult *P. pugio* and adult *I. obsoleta* were collected from Leadenwah Creek (N 32°38'51.00"; W 080°13'18.05'), a tidal tributary of the North Edisto River, SC. *P. pugio* embryos were removed from ovigerous females under microscopes. *P. pugio* larvae were obtained by placing ovigerous adult shrimp in brooding containers within 10-L aquaria. The brooding containers were designed to allow the embryos to hatch and the larvae to escape through the mesh. Adult mud snails deposited egg capsules on the glass sides of the aquarium. Egg capsules were collected and transferred to glass finger bowls containing filtered (0.22 µm) 20 ppt seawater until larvae hatched. Larval and juvenile clams, *M. mercenaria*, were acquired from Bay Shellfish, Inc. (Terra Ceia, FL). Juvenile *N. arenaceodentata* were obtained from Aquatic Toxic Support (Bremerton, WA).

Seawater (for all testing) was acquired from Charleston Harbor estuary (N 32°45'11.52"; W 79°53'58.31"), prefiltered (5 µm), activated carbon filtered, and diluted with deionized water to adjust salinity to 20 ppt. Seawater for the mysid test was UV-sterilized and further filtered to 1 µm. Seawater for the grass shrimp embryo test, the larval clam, and larval snail tests was further filtered to 0.22 µm.

Dispersant Testing

All species were tested with Corexit and Finasol, individually, using static renewal exposures. Every 24 h, dead animals were removed, water quality (temperature, salinity, pH, and dissolved oxygen) was assessed, and the test solutions were renewed. Test chambers consisted of glass jars or beakers (covered and aerated) or 24-well polystyrene plates coated with hydrogel (Corning™) to reduce chemical adherence (Chandler et al. 2004) placed on an orbital shaker (80 rpm). Range finding assays were conducted to determine appropriate dispersant exposure concentrations. For each species and life stage, a definitive test consisting of a seawater control and five nominal concentrations was conducted to determine a median lethal concentration (LC₅₀) for both Corexit and Finasol. Additional test conditions for each species are provided in Table 1.

Table 1

Bioassay parameters for each species and life stage tested

Species/life stage	Age/size	Test duration	# Individuals per test chamber/# replicates	Volume in test chamber	Temp (°C)	Photoperio
<i>C. variegatus</i> (fish) embryos	48 h after fertilization	9 days	15/4	300 mL	25	16 h L:8 h I
<i>C. variegatus</i> adult	3.9–5.0 cm	96 h	6/3	3.5 L	25	16 h L:8 h I
<i>P. pugio</i> (shrimp) embryo	Stage VI	96 h	24 per well plate/3	2 mL per well	28	24 h dark
<i>P. pugio</i> larvae	24–48-h-old	96 h	10/3	400 mL	25	16 h L:8 h I
<i>P. pugio</i> adult	2–3 cm	96 h	10/3	2L	25	16 h L:8 h I
<i>M. mercenaria</i> (clam) larvae	7 days old	96 h	24 per well plate/3	2 mL per well	25	16 h L:8 h I
<i>M. mercenaria</i> juvenile	1.0–1.2 mm	96 h	30/3	180 mL	25	16 h L:8 h I
<i>I. obsoleta</i> (snail) larvae	≤24-h-old	96 h	24 per well plate/3	2 mL per well	25	16 h L:8 h I
<i>I. obsoleta</i> adult	15–18 mm	96 h	10/3	400 mL	25	16 h L:8 h I
<i>L. plumulosus</i> (amphipod) juvenile	500–710 μm	96 h	10/3	80 mL	25	16 h L:8 h I
<i>N. arenaceodentata</i> (polychaete) juvenile	~2-weeks-old, 10–15 mm	96 h	5/3	300 mL	25	16 h L:8 h I
<i>A. bahia</i> (mysid)	5-days-old	48 h	10/3	400 mL	25	16 h L:8 h I

Dispersed Oil (CEWAF) Testing

Each of the 12 bioassays (fish: embryo-larval and adult; shrimp: embryo, larval, and adult; clam: larvae and juvenile; snail: larvae and adult; mysid; polychaete; and amphipod) performed with the individual dispersants were repeated using CEWAFs of the dispersants in mixture with Louisiana Sweet Crude (LSC) oil. Preparation of the CEWAFs followed methods similar to Hemmer et al. (2011), using low-energy mixing (vortex 25% of the solution height, stirred for 18 h and allowed to sit for 6 h). Each CEWAF consisted of 19 L of 20 ppt seawater, 25 g/L of oil, and 1.25 g of dispersant/L (a ratio of 1:20 dispersant:oil). The 100% CEWAF was diluted with 20 ppt seawater to achieve additional treatments (50, 16.7, 5.6, 1.85, 0.62 and 0.21%). Controls consisted of 20 ppt seawater. Test methods were similar to those used for the individual dispersant testing, except that CEWAF testing was conducted using static exposures (Hemmer et al. 2011).

Cellular Bioassays

Sublethal effects on cellular function measured from surviving animals at the end of the 96 h exposure included cytochrome p450 enzyme induction and splenocyte proliferation activity in adult fish; and acetylcholinesterase activity and lipid peroxidation in adult fish, adult shrimp, and adult snails. Cytochrome p450 enzyme induction [based on ethoxyresorufin (EROD)] was measured as an indication of hydrocarbon metabolism in adult fish from the individual dispersants tests and the CEWAF tests. Microsomal fractions of the livers were obtained and measurement of enzyme activity and protein concentration were performed simultaneously using a well plate format adapted from Kennedy and Jones (1994) and DeLorenzo et al. (2012). The splenocyte proliferation assay was performed according to Parent et al. (2011), as a measure of fish immunotoxicity. Assessment of lipid peroxidation activity (LPX) as a measure of cellular oxidative damage was performed for adult fish (liver tissue), adult grass shrimp (whole shrimp), and adult mud snails (tissue removed from shell) from the dispersant alone and CEWAF exposures according to the malondialdehyde method of Ringwood et al. (2003), adapted to microplate format (DeLorenzo et al. 2006). As a measure of nervous system function, acetylcholinesterase (AChE) enzyme activity was assessed using methods of Key et al. (1998) in adult fish brain tissue from the CEWAF exposures and adult grass shrimp (whole shrimp) and adult snails (tissue removed from shell) from dispersant alone and CEWAF exposures.

Chemical Analysis

The different exposure protocols (static-renewal for dispersant alone testing and static for CEWAF testing) were selected based on available chemical analyses. Dispersant solutions were renewed to maintain a relatively constant chemical concentration throughout the test, because we could not chemically analyze the products. The dispersant-only testing is reported as nominal concentrations of Finasol and Corexit. The CEWAF solutions were not renewed, consistent with methods in Hemmer et al. (2011), but were chemically analyzed to assess hydrocarbon concentrations. The chemical analyses conducted for the CEWAF testing included Total PAH, which was based on a suite of 50 parent and alkylated PAHs (Supplemental Table 1), and total extractable hydrocarbons (TEH) sampled at time (t) = 0 (immediately after dosing) and at $t = 6$ h, $t = 24$ h, and $t = 96$ h for the large volume toxicity tests (adult fish and adult grass shrimp). A time weighted average (TWA) concentration was calculated for total PAH and TEH using the equation: $TWA = (t_1c_1 + t_2c_2 + t_3c_3 + t_4c_4)/(t_1 + t_2 + t_3 + t_4)$, where t = time and c = concentration at each sampling point. The sample taken immediately after dosing was considered 1 h, such that the denominator of the equation = 127 h. Water samples (50–500 mL) were collected from each CEWAF test chamber, and replicate samples were composited by treatment and analyzed for TEH and total PAH according to NOAA SOP CCR-052 (Supplemental Data).

Statistical Analysis

All median lethal concentrations (LC_{50} values) with 95% confidence intervals (CIs), as well as the 10% effect concentration (LC_{10} values) were determined using SAS Probit Analysis (PROC PROBIT, SAS V.9.4, Cary, NC). The program analyzed data with and without log transformation using a normal, logistic, or Weibull distribution and the best-fit model was selected. Dispersant LC_{50} and LC_{10} values were calculated using nominal exposure concentrations due to the proprietary nature of the Finasol product. The CEWAF LC_{50} and LC_{10} values were calculated using nominal concentrations (% CEWAF) and then recalculated using the measured chemistry values (mg/L) for TEH and total PAH. Significant differences ($p < 0.05$) between LC_{50} s of the different chemicals and life stages were determined using the LC_{50} ratio test (Wheeler et al. 2006). Statistical differences among treatments were determined using analysis of variance (ANOVA). Where ANOVA revealed a significant difference among treatments ($p < 0.05$), Dunnett's procedure for multiple comparisons was used to determine which treatments differed significantly from the control.

Results

Water quality for all toxicity tests was maintained within acceptable ranges for dissolved oxygen ($\geq 60\%$ saturation), pH (8.0 ± 0.5), temperature ($25\text{ }^{\circ}\text{C} \pm 2$), and salinity ($20\text{ ppt} \pm 2$). Control survival for all definitive tests met protocol standards ($>80\%$ fish and shrimp embryo tests; $>90\%$ all other tests). For the majority of the test species, Corexit and Finasol exposures were performed concurrently.

Exceptions are the adult shrimp, larval clam, and juvenile clam tests. Corexit and Finasol CEWAFs were tested concurrently, with the exception of the adult fish, amphipods, and polychaetes. Tests were repeated if the concentration range was either too high or too low to yield a 50% effect concentration. Reference tests using sodium dodecyl sulfate were performed to verify uniformity of response for each batch of field collected test organisms.

Dispersant Toxicity

Table 2 summarizes the dispersant treatments that caused significant mortality in each species. Toxicity values (LC_{10} and LC_{50}) for each species and life stage were calculated using nominal concentrations and are ranked in order of sensitivity (for Corexit) (Table 3). The data indicate that larval life stages were generally more sensitive than adult life stages for the same species and that Finasol, in general, had significantly greater toxicity (approximately fourfold higher) to the estuarine test species than Corexit (Table 3). The mean LC_{50} value for all species tested with Corexit was 150.31 mg/L (range 9.85–702.41), whereas the mean LC_{50} value for all species tested with Finasol was 43.27 mg/L (range 3.81–105.26).

Table 2

Average percent mortality at each nominal exposure concentration for the Corexit and Finasol LC_{50} tests

Species, life stage, exposure duration	Corexit nominal (mg/L)	Average percent Mortality	Finasol nominal (mg/L)	Average percent Mortality
Fish, ELS	12	28	12	27
9 days	37	30	37	28
	111*	33	111*	93
	333*	95	333*	100
	1000*	100	1000*	97
Fish, adult	12	0	12	0

Species, life stage, exposure duration	Corexit nominal (mg/L)	Average percent Mortality	Finasol nominal (mg/L)	Average percent Mortality
96 h	37*	11	37	0
	111*	17	111*	94
	333*	100	333*	100
	1000*	100	1000*	100
Shrimp, embryo	12	24	12	24
96 h	37	26	37	32
	111*	33	111*	56
	333*	51	333*	75
	1000*	64	1000*	81
Shrimp, larvae	4	7	4	0
96 h	8	13	8	10
	16*	23	16*	33
	32*	17	32*	97
	64*	33	64*	100
Shrimp, adult	12	0	12	23
96 h	37	0	37*	43
	111	13	111*	80
	333*	27	333*	100
	1000*	60	1000*	100
Snail, larvae	1.4	14	1.4*	21
96 h	4.1*	22	4.1*	53
	12*	52	12*	100
	37*	96	37*	100
	111*	100	111*	100
Snail, adult	12	0	12	0
96 h	37	0	37	0
	111*	27	111*	77

Species, life stage, exposure duration	Corexit nominal (mg/L)	Average percent Mortality	Finasol nominal (mg/L)	Average percent Mortality
	333*	93	333*	83
	1000*	100	1000*	100
Clam, larvae	1.4*	21	1.6	14
96 h	4.1*	39	3.1	10
	12*	49	6.3*	18
	37*	76	13*	44
	111*	100	25*	69
Clam, juvenile	1.4	18	1.6	11
96 h	4.1*	26	3.1*	36
	12*	29	6.3*	53
	37*	33	13*	98
	111*	100	25*	100
Polychaete, juv.	12	0	1.4	0
96 h	37	0	4.1	0
	111*	93	12	0
	333*	100	37	6.7
	1000*	100	111*	100
Amphipod, juv.	12	13	1.4	0
96 h	37*	97	4.1	0
	111*	100	12*	10
	333*	100	37*	100
	1000*	100	111*	100
Mysid, juvenile	4.1	3.3	4.1	3.3
48 h	12	6.7	12	3.3
	37*	67	37*	100
	111*	100	111*	100
	333*	100	333*	100

Species, life stage, exposure duration	Corexit nominal (mg/L)	Average percent Mortality	Finasol nominal (mg/L)	Average percent Mortality
Asterisks indicate concentrations that were significantly different from the control (ANOVA, followed by Dunnett's test)				

Table 3

LC₅₀ (95% confidence interval), and LC₁₀ toxicity values determined for Corexit and Finasol for each test species

Species, life stage	Corexit	Corexit	Corexit	Finasol	Finasol	Finasol
	LC ₅₀	95% CI	LC ₁₀	LC ₅₀	95% CI	LC ₁₀
Snail, larvae	9.85*	(7.96–11.92)	1.58	3.81*	(3.26–4.60)	0.61
Clam, larvae	16.10	(12.00–20.73)	<1.37	15.30	(12.65–18.72)	2.21
Amphipod, juv.	21.43	(17.34–26.07)	10.55	22.59	(18.37–28.15)	9.57
Mysid, juvenile	32.80*	(28.46–37.02)	15.54	24.95*	(19.40–32.74)	15.09
Clam, juvenile	43.40*	(36.81–51.00)	<1.37	5.47*	(4.46–6.29)	<1.56
Shrimp, larvae	64.05*	(54.90–74.16)	7.57	18.65*	(16.04–22.15)	9.61
Polychaete, juv.	101.24*	(94.32–108.66)	93.77	40.85*	(37.55–44.24)	37.68
Fish, ELS	142.26*	(111.50–171.54)	<12.3	37.14*	(20.68–60.29)	8.29
Snail, adult	153.99	(124.76–190.35)	80.76	105.26	(67.17–157.18)	39.07
Fish, adult	162.66	(124.85–237.58)	68.47	105.04	(102.40–107.75)	97.67
Shrimp, embryo	353.51*	(180.10–836.11)	<12.3	85.99*	(44.00–157.89)	2.98
Shrimp, adult	702.41*	(471.50–1308.47)	122.34	54.17*	(34.65–75.48)	<12.3

Toxicity values were calculated using nominal exposure concentrations (mg/L)

Asterisks indicate a significant difference between Corexit and Finasol LC₅₀ values (Wheeler ratio test $p < 0.05$)

Sublethal Effects *C. variegatus*

There was no significant effect of either dispersant on fish EROD activity (ANOVA Corexit $p = 0.4863$; ANOVA Finasol $p = 0.2598$). Mean EROD activity (μM resorufin/mg protein) [\pm standard error (SE)] in the treatments ranged from 0.404 (± 0.088) to 0.889 (± 0.166). Lipid peroxidation activity in adult *C. variegatus* was also not significantly affected by dispersant exposure (ANOVA Corexit $p = 0.6276$; ANOVA Finasol $p = 0.1708$) and the mean MDA levels (nmol/g wet weight) across treatments ranged from 189.79 (± 125.94) to 488.63 (± 268.62). Fish tissues from the dispersant-only tests were not preserved for AChE activity or splenocyte proliferation.

Finasol exposure significantly impaired *C. variegatus* embryonic hatching success ($p < 0.0001$). Nominal Finasol concentrations of 333 and 1000 mg/L reduced hatching success by 50 and 80%, respectively, compared to controls. Embryos exposed to 1000 mg/L of Finasol took significantly longer to hatch than controls ($p < 0.0001$): 7 days versus 5 days. No effects on hatching success ($p = 0.2055$) or time-to-hatch ($p = 0.2144$) occurred in the Corexit exposures.

Sublethal Effects *P. pugio*

P. pugio embryo hatching success was significantly reduced in nominal Corexit concentrations ≥ 37 mg/L (ANOVA $p = 0.0012$; $>85\%$ reduction in hatching success compared to control) and in nominal Finasol concentrations ≥ 111 mg/L (ANOVA $p < 0.0001$; $>79\%$ reduced hatching success compared with control). Finasol (111 mg/L) significantly increased lipid peroxidation activity in adult grass shrimp compared to control levels; indicating a negative effect on cellular membranes. Corexit (1000 mg/L, nominal concentration) also significantly increased lipid peroxidation activity in adult grass shrimp. Larval grass shrimp were the most sensitive *P. pugio* life stage tested for both dispersants. Embryos were the least sensitive life stage tested for both dispersants. Acetylcholinesterase activity of Corexit- or Finasol-exposed grass shrimp was not significantly different from controls (ANOVA $p = 0.2008$) and ranged from mean AChE (nmol/min) (\pm SE) of 0.2145 (± 0.0135) to 0.2326 (± 0.0282).

Sublethal Effects *I. obsoleta*

There was no significant effect from either dispersant alone on lipid peroxidation activity in adult mud snails (ANOVA Corexit $p = 0.9728$; ANOVA Finasol $p = 0.0929$). The mean MDA levels (nmol/g wet weight) across treatments ranged

from 96.15 (± 5.91) to 100.09 (± 14.56) in the Corexit exposure and from 72.43 (± 4.51) to 88.93 (± 6.20) in the Finasol exposure.

Nominal Finasol concentrations ≥ 111 mg/L caused significant acetylcholinesterase inhibition in adult *I. obsoleta* ($p = 0.0018$). Mean AChE activity was reduced 32–51% in the Finasol treatments compared to control levels. There was no significant effect of Corexit on acetylcholinesterase activity in adult mud snails ($p = 0.0627$) and mean AChE (nmol/mgP/min) (\pm SE) ranged from 85.05 (± 8.60) to 107.33 (± 4.90) across treatments.

Dispersed Oil (CEWAF) Toxicity

CEWAF treatments that caused significant mortality in each species are summarized in Table 4. Toxicity values (LC_{10} and LC_{50}) for each species were calculated using nominal percent CEWAF concentrations and ranked in order of sensitivity for Corexit-CEWAF (Table 5). The nominal percent CEWAF data indicate that larval life stages were generally more sensitive than adult life stages for the same species and that Corexit-CEWAF had greater toxicity to the estuarine test species than Finasol-CEWAF (Table 5). Toxicity values were then calculated using the measured chemical concentrations for TEH (Table 6) and Total PAH (Table 7), demonstrating greater toxicity for Finasol-CEWAF than for Corexit-CEWAF. Using measured TEH concentrations, the mean LC_{50} value for all species tested with Corexit-CEWAF was 261.96 mg/L, whereas the mean LC_{50} value for all species tested with Finasol-CEWAF was 23.19 mg/L. Similarly, using measured total PAH concentrations, the mean LC_{50} value for all species tested with Corexit-CEWAF was 2.95 mg/L, whereas the mean LC_{50} value for all species tested with Finasol-CEWAF was 0.49 mg/L.

Table 4

Average percent mortality at each nominal exposure concentrations for the CEWAF LC_{50} tests

Species, life stage, exposure duration	Corexit	Average	Finasol	Average
	CEWAF (%)	percent Mortality	CEWAF (%)	percent Mortality
Fish, ELS	1.85	21.67	1.85	13.33
9 days	5.56	26.67	5.56	25
	16.7*	98.33	16.7	30
	50*	100	50	23.33

Species, life stage, exposure duration	Corexit	Average	Finasol	Average
	CEWAF (%)	percent Mortality	CEWAF (%)	percent Mortality
	100*	100	100*	100
Fish, adult	1.85	11.11	1.85	0
96 h	5.56	0	5.56	0
	16.7	22.22	16.7	0
	50*	100	50*	11.11
	100*	100	100*	100
Shrimp, embryo	1.85	2.78	1.85	4.17
96 h	5.56	4.17	5.56	2.78
	16.7	19.44	16.7	11.11
	50	18.06	50*	48.61
	100*	36.11	100*	62.50
Shrimp, larvae	0.62	0	0.62	0
96 h	1.85	3.33	1.85	0
	5.56*	73.33	5.56	3.33
	16.7*	100	16.7*	96.67
	50*	100	50*	100
Shrimp, adult	1.85	3.33	1.85	0
96 h	5.56*	40	5.56	3.33
	16.7*	93.33	16.7	6.67
	50*	100	50*	36.67
	100*	100	100*	93.33
Snail, larvae	0.069	31.94	1.85*	43.06
96 h	0.20	37.50	5.56*	84.72
	0.62*	38.89	16.7*	100.00
	1.85*	50.00	50*	100.00
	5.56*	94.44	100*	100.00

Species, life stage, exposure duration	Corexit	Average	Finasol	Average
	CEWAF (%)	percent Mortality	CEWAF (%)	percent Mortality
Snail, adult	1.85	0.00	1.85	0.00
96 h	5.56	0.00	5.56	0.00
	16.7*	20.00	16.7*	6.67
	50*	100.00	50*	100.00
	100*	100.00	100*	100.00
Clam, larvae	0.62	11.11	0.62	1.39
96 h	1.85*	83.33	1.85	15.28
	5.56*	80.56	5.56	4.17
	16.7*	98.61	16.7*	100
	50*	91.67	50*	93.06
Clam, juvenile	0.2*	12.22	0.2	6.67
96 h	0.62*	18.89	0.62*	18.89
	1.85*	34.44	1.85*	24.44
	5.56*	44.44	5.56*	33.33
	16.7*	85.56	16.7*	48.89
Polychaete, juv.	1.85	0.00	1.85	0.00
96 h	5.56	0.00	5.56	0.00
	16.7*	86.67	16.7	6.67
	50*	100.00	50*	93.33
	100*	100.00	100*	100.00
Amphipod, juv.	0.2	10	0.62	0
96 h	0.62	13.33	1.85	3.33
	1.85*	10.00	5.56	3.33
	5.56*	63.33	16.7*	30
	16.7*	100.00	50*	100
Mysid, juvenile	0.62	0	0.62	0.00

Species, life stage, exposure duration	Corexit	Average	Finasol	Average
	CEWAF (%)	percent Mortality	CEWAF (%)	percent Mortality
48 h	1.85*	33.33	1.85	0.00
	5.56*	66.67	5.56	6.67
	16.7*	100.00	16.7*	80.00
	50*	100.00	50*	100.00

Asterisks indicate concentrations that were significantly different from the control (ANOVA, followed by Dunnett's test)

Table 5

LC₅₀ (95% confidence interval) and LC₁₀ toxicity values for Corexit and Finasol CEWAFs determined for each test species

Species, life stage	Corexit	Corexit	Corexit	Finasol	Finasol	Finasol
	LC ₅₀	95% CI	LC ₁₀	LC ₅₀	95% CI	LC ₁₀
Clam, larvae	1.29*	(0.33–2.67)	0.11	8.34*	(4.77–14.43)	2.15
Snail, larvae	1.61*	(1.12–2.07)	<0.069	2.49*	(1.18–3.25)	<1.85
Amphipod, juv.	2.59*	(1.35–3.99)	0.33	19.91*	(16.7–28.39)	10.71
Mysid, juvenile	3.21*	(2.47–4.15)	1.15	11.22*	(9.13–13.72)	6.19
Shrimp, larvae	4.59*	(3.63–5.23)	2.63	9.63*	(7.93–11.69)	6.56
Clam, juvenile	7.30*	(6.18–8.62)	0.33	16.50*	(13.05–21.45)	0.46
Fish, ELS	7.72*	(6.38–9.08)	0.63	50.68*	(39.01–64.22)	0.81
Shrimp, adult	8.38*	(6.85–10.38)	2.23	59.45*	(51.31–69.55)	25.95
Polychaete, juv.	14.64*	(7.50–22.94)	4.18	28.85*	(21.72–38.14)	18.08
Snail, adult	21.89	(17.77–56.42)	13.10	27.33	(22.79–33.30)	17.92

Species, life stage	Corexit	Corexit	Corexit	Finasol	Finasol	Finasol
	LC	95% CI	LC	LC	95% CI	LC
Fish, adult	26.73*	(19.68–39.45)	6.50	67.54*	(57.71–79.96)	45.26
Shrimp, embryo	128.36*	(89.49–275.39)	15.98	65.87*	(49.88–91.39)	9.83

Toxicity values were calculated using nominal exposure concentrations (% CEWAF) This sentence needs to be moved into the Table heading. ...

Asterisks indicate a significant difference between Corexit and Finasol CEWAF LC₅₀ values (Wheeler ratio test $p < 0.05$)

Table 6

LC₅₀ (95% confidence interval) and LC₁₀ toxicity values for Corexit and Finasol CEWAFs determined for each test species

Species, life stage	Corexit	Corexit	Corexit	Finasol	Finasol	Finasol
	LC ₅₀	95% CI	LC ₁₀	LC ₅₀	95% CI	LC ₁₀
Snail, larvae	10.39	(6.68–13.85)	<2.45	0.68	(0.004–1.48)	<3.90
Clam, larvae	10.80	(2.48–22.55)	0.89	7.77	(1.12–5.78)	0.98
Amphipod, juv.	20.22	(10.41–32.25)	2.13	6.45	(5.21–9.65)	2.91
Mysid, juvenile	37.28	(28.03–49.42)	11.50	13.05	(10.96–15.34)	6.44
Shrimp, larvae	64.88	(51.27–73.85)	37.16	13.11	(10.79–15.92)	8.93
Clam, juvenile	84.61	(71.62–99.69)	<2.90	7.38	(5.87–9.34)	<2.10
Shrimp, adult	105.40	(85.07–131.96)	23.70	26.17	(23.18–28.97)	11.92
Polychaete, juv.	126.31	(78.97–176.58)	59.16	12.30	(9.29–15.78)	6.70
Fish, ELS	127.97	(102.91–153.78)	<7.50	46.72	(34.21–59.41)	<4.40
Snail, adult	225.05	(178.89–592.76)	126.88	26.70	(22.00–32.25)	15.26

Species, life stage	Corexit	Corexit	Corexit	Finasol	Finasol	Finasol
	LC	95% CI	LC	LC	95% CI	LC
Fish, adult	515.56	(413.69–637.34)	169.27	28.21	(24.49–32.25)	20.67
Shrimp, embryo	1815.03	(1265.32–3894.26)	225.90	89.72	(67.93–124.47)	13.39

Toxicity values were calculated using measured TEH concentrations (mg/L) This sentence needs to be moved into the Table heading. ...

Table 7

LC₅₀ (95% confidence interval) and LC₁₀ toxicity values for Corexit and Finasol CEWAFs determined for each test species

Species, life stage	Corexit	Corexit	Corexit	Finasol	Finasol	Finasol
	LC ₅₀	95% CI	LC ₁₀	LC ₅₀	95% CI	LC ₁₀
Clam, larvae	0.12	(0.03–0.26)	0.01	0.10	(0.06–0.19)	0.03
Snail, larvae	0.30	(0.21–0.39)	<0.01	0.03	(0.01–0.04)	<0.02
Clam, juvenile	0.64	(0.54–0.75)	<0.01	0.09	(0.06–0.13)	0.01
Shrimp, larvae	0.64	(0.51–0.72)	0.37	0.17	(0.14–0.21)	0.12
Amphipod, juv.	0.65	(0.36–0.93)	0.11	0.26	(0.19–0.53)	0.11
Mysid, juvenile	0.81	(0.63–0.97)	0.28	0.18	(0.15–0.22)	0.10
Fish, ELS	1.24	(1.08–1.39)	<0.18	1.11	(0.73–1.68)	<0.01
Shrimp, adult	1.44	(1.21–1.76)	0.60	1.25	(0.82–1.68)	0.41
Snail, adult	2.34	(1.90–6.09)	1.39	0.34	(0.28–0.43)	0.22
Polychaete, juv.	2.68	(1.60–3.76)	1.03	0.40	(0.27–0.57)	0.21
Fish, adult	6.96	(5.55–8.62)	2.20	0.66	(0.56–0.78)	0.44

Species, life stage	Corexit	Corexit	Corexit	Finasol	Finasol	Finasol
	LC	95% CI	LC	LC	95% CI	LC
Shrimp, embryo	17.80	(12.41–38.20)	2.22	1.14	(0.86–1.60)	0.17

Toxicity values were calculated using measured Total PAH concentrations (mg/L) This sentence needs to be moved into the Table heading.

Sublethal Effects *C. variegatus*

Exposure to Corexit and Finasol CEWAFs did not significantly alter fish immune function as measured by splenocyte proliferation (ANOVA $p = 0.3876$). Mean splenocyte cell density (fluorescent units [FU] = fluorescence at 485/530 nm) (\pm SE) ranged from 4462.67 FU (\pm 417.23) to 9510.44 FU (\pm 3017.40) across treatments. CEWAF exposure also did not have a significant effect on nervous system function as measured by brain acetylcholinesterase activity (Corexit-CEWAF $p = 0.6205$; Finasol-CEWAF $p = 0.2869$). Mean AChE (nmol/mgP/min) (\pm SE) in the treatments ranged from 233.87 (\pm 103.25) to 354.55 (\pm 40.03).

There was a significant increase in EROD activity for fish exposed to 5.56 and 16.7% Finasol-CEWAF concentrations (ANOVA $p = 0.0032$) and 1.85% Corexit-CEWAF (ANOVA $p = 0.0360$) compared with the control. Mean activity (μ mol/min/ μ g protein) increased up to fivefold in the Finasol-CEWAF treatments compared with the control, and a maximum ninefold induction of enzyme activity compared with control was observed in the Corexit-CEWAF exposure.

There was a significant decreasing effect on lipid peroxidation activity in the CEWAF exposed fish to \geq 1.85% Corexit-CEWAF and to \geq 16.7% Finasol-CEWAF ($p = 0.0023$). Mean LPX (nmol MDA/mg wet weight) (\pm SE) in the CEWAF treatments ranged from 847.99 (\pm 163.12) in the controls to 105.86 (\pm 116.46) in the highest Finasol-CEWAF concentration and 210.32 (\pm 168.81) in the highest Corexit-CEWAF concentration.

Only the 100% Finasol-CEWAF negatively impacted embryonic hatching success (7% hatch vs. 93% in the controls; $p < 0.0001$) and embryos exposed to 100% Finasol-CEWAF took significantly longer to hatch than controls (mean hatch time of 9 vs. 5.5 days in the controls; $p < 0.0001$). Embryos exposed to Corexit-CEWAF \geq 16.7% had significantly reduced hatching success ($p < 0.0001$) and significantly delayed time-to-hatch ($p < 0.0001$). Hatching success was reduced from 90% in the

controls to 62, 23, and 3% in the 16.7, 50, and 100% Corexit-CEWAF treatments, respectively. Embryos that hatched in the 50 and 100% CEWAFs were not viable (larvae died shortly after hatching). Mean time to hatch increased from 5.5 days in the controls to 9 days in the 50 and 100% Corexit-CEWAFs.

Sublethal Effects *P. pugio*

Grass shrimp embryo hatching success was significantly lower in Corexit and Finasol CEWAF exposures (ANOVA p values <0.0001). Mean hatching success declined from 94% in the controls to 83, 61, and 14% in the Corexit-CEWAF concentrations of 1.85, 5.56, and 16.7%, respectively, and from 97% in the controls to 76, 74, 74, and 61% in the Finasol-CEWAF concentrations of 0.62, 1.85, 5.56, and 16.7%, respectively. None of the embryos in the 50 and 100% dispersant CEWAFs hatched.

There was no significant effect of Corexit-CEWAF ($p = 0.3584$) or Finasol-CEWAF ($p = 0.6400$) on grass shrimp acetylcholinesterase activity. Mean AChE (nmol/mgP/min) (\pm SE) for all treatments ranged from 51.26 (\pm 3.24) to 74.94 (\pm 12.29). There also was no significant effect on lipid peroxidation activity in adult grass shrimp exposed to Corexit-CEWAF ($p = 0.2116$) or Finasol-CEWAF ($p = 0.5472$), although there was a trend toward increasing activity. Mean LPX (nmol MDA/mg wet weight) (\pm SE) ranged from 206.18 (\pm 122.34) to 507.16 (\pm 186.16) in the Finasol-CEWAF and ranged from 338.77 (\pm 96.54) to 593.58 (\pm 196.93) in the Corexit-CEWAF.

Sublethal Effects *I. obsoleta*

There was no significant effect on lipid peroxidation activity in adult mud snails exposed to Finasol-CEWAF ($p = 0.1880$), and mean LPX (nmol MDA/mg wet weight) (\pm SE) ranged from 66.33 (\pm 27.89) in the control to 104.11 (\pm 11.39) in the highest treatment. Mean LPX activity in the 16.7% Corexit-CEWAF (29.08 ± 6.63) was significantly lower than controls (62.90 ± 15.17 ; $p = 0.0286$). There was no significant effect of Corexit-CEWAF ($p = 0.7997$) or Finasol-CEWAF ($p = 0.1134$) on adult snail acetylcholinesterase activity. Mean AChE (nmol/mgP/min) (\pm SE) in the treatments ranged from 70.34 (\pm 6.55) to 109.28 (\pm 7.31).

Measured CEWAF Concentrations

Measured chemistry in the CEWAFs included TEH and Total PAH (list of 50 parent and alkylated PAH analytes provided in Supplemental Table 1). Addition of both dispersants to LSC oil chemically enhanced the petroleum signatures detected in

the water-accommodated fractions (WAFs). In laboratory testing with undispersed LSC WAFs before this study, TEH and measured individual and alkylated PAH concentrations were below detection limits; detection limits were 2 mg/L for TEH and ranged from $5 \times 10^{-7} - 0.107$ mg/L for PAHs in the Total PAH. Minimum detection levels were calculated according to Ragland et al. (2014). The $t = 0$ measured TEH concentrations (mean \pm SE) in this study were 1315 ± 242 mg/L in the 100% Corexit-CEWAFs and 67.2 ± 11.0 mg/L in the 100% Finasol-CEWAFs (Table 8). Concentrations of TEH were significantly higher in the Corexit-CEWAFs than the Finasol-CEWAFs ($p = 0.0019$). Total PAH concentrations (mean \pm SE) measured in this study were 14.2 ± 1.32 mg/L in the 100% Corexit-CEWAFs and 1.44 ± 0.10 mg/L in the 100% Finasol-CEWAFs (Table 8), and total PAH concentrations in the Corexit-CEWAFs also were significantly higher than in the Finasol-CEWAFs ($p < 0.0001$).

Table 8

Measured TEH and Total PAH concentrations (mg/L) in the Corexit CEWAF and Finasol CEWAF for each toxicity test at $t = 0$

Species, life stage	Corexit % CEWAF	Corexit CEWAF TEH (mg/L)	Corexit CEWAF Total PAH (mg/L)	Finasol % CEWAF	Finasol CEWAF TEH (mg/L)	Finasol CEWAF Total PAH (mg/L)
Fish, ELS	0.62	7.50	0.18	0.62	0.00	0.01
	1.85	18.5	0.45	1.85	0.00	0.04
	5.56	78.8	1.20	5.56	4.40	0.09
	16.7	300	2.20	16.7	17.5	0.26
	50	872	8.20	50	49.0	1.42
	100	1704	13.0	100	83.0	1.54
Fish, adult	0.62	9.90	0.18	0.62	0.00	0.00
	1.85	27.3	0.51	1.85	0.00	0.00
	5.56	154	1.04	5.56	2.10	0.04
	16.7	397	5.48	16.7	7.80	0.12
	50	850	11.6	50	21.0	0.45
	100	2892	20.2	100	37.6	0.94
Shrimp, embryo	0.62	8.73	0.09	0.62	0.84	0.01

Species, life stage	Corexit % CEWAF	Corexit CEWAF TEH (mg/L)	Corexit CEWAF Total PAH (mg/L)	Finasol % CEWAF	Finasol CEWAF TEH (mg/L)	Finasol CEWAF Total PAH (mg/L)
	1.85	26.2	0.26	1.85	2.52	0.03
and	5.56	78.6	0.77	5.56	7.57	0.10
	16.7	236	2.31	16.7	22.7	0.29
Shrimp, larvae	50	707	6.93	50	68.1	0.87
	100	1414	13.87	100	136	1.73
Shrimp, adult	0.62	3.70	0.17	0.62	0.00	0.01
	1.85	18.1	0.49	1.85	0.00	0.04
	5.56	67.6	1.17	5.56	0.00	0.13
	16.7	216	2.61	16.7	7.50	0.36
	50	550	7.79	50	21.7	1.58
	100	1150	16.6	100	37.0	1.63
Snail, larvae	0.07	0.00	0.01	0.62	0.00	0.01
	0.20	0.00	0.03	1.85	0.00	0.02
	0.62	2.45	0.10	5.56	3.90	0.07
	1.85	11.0	0.39	16.7	12.4	0.20
	5.56	40.8	1.01	50	43.4	0.67
	100	664	19.2	100	74.6	1.44
Snail, adult	0.62	6.85	0.06	0.62	0.00	0.01
	1.85	13.5	0.17	1.85	0.00	0.02
and	5.56	38.6	0.59	5.56	3.90	0.07
	16.7	167	1.78	16.7	12.4	0.20
Clam larvae	50	451	4.90	50	43.4	0.67
	100	589	8.82	100	74.6	1.44
Clam, juvenile	0.20	2.90	0.01	0.20	0.00	0.00

Species, life stage	Corexit % CEWAF	Corexit CEWAF TEH (mg/L)	Corexit CEWAF Total PAH (mg/L)	Finasol % CEWAF	Finasol CEWAF TEH (mg/L)	Finasol CEWAF Total PAH (mg/L)
	0.62	9.00	0.05	0.62	0.00	0.00
	1.85	23.1	0.16	1.85	0.00	0.00
	5.56	55.9	0.55	5.56	2.10	0.04
	16.7	200	1.41	16.7	7.80	0.12
	50	374	4.65	50	21.0	0.45
	100	718	9.10	100	37.6	0.94
Mysid, juvenile	0.62	7.50	0.18	0.62	0.00	0.01
	1.85	18.5	0.45	1.85	0.00	0.04
	5.56	78.8	1.18	5.56	4.40	0.09
	16.7	300	2.19	16.7	17.5	0.26
	50	872	8.15	50	49.0	1.42
	100	1704	13.0	100	83.0	1.54
Polychaete, juv	0.62	4.20	0.14	0.62	0.00	0.02
	1.85	14.6	0.56	1.85	0.00	0.05
and	5.56	43.2	1.28	5.56	0.00	0.08
	16.7	185	2.99	16.7	5.20	0.19
Amphipod, juv.	50	456	6.82	50	19.0	0.84
	100	1000	14.1	100	41.2	1.73
CEWAF preparations were used for more than one toxicity test, thus species are reported together based on common CEWAFs						

The hydrocarbon concentrations measured over time in the large volume tests for Corexit-CEWAF and Finasol-CEWAF are shown in Tables 9 and 10, respectively. The TEH concentrations in the Corexit-CEWAF treatments were 61 and 22% of the initial concentrations after 24 h and 96 h, respectively (mean of all Corexit-CEWAF treatments in Table 9). Similarly, the Total PAH concentrations in the Corexit-CEWAF treatments were 43 and 16% of the initial concentrations after 24 h and

96 h, respectively. Measured hydrocarbon degradation over time was more variable in the Finasol-CEWAF treatments because of limits in detecting the lower concentrations that were present in the Finasol-CEWAFs at the start of the test (Table 10). TEH concentrations in the Finasol-CEWAF treatments averaged 56% of the initial concentrations after 24 h but were undetectable after 96 h (Table 10). The total PAH concentrations in the Finasol-CEWAF treatments were 14 and 3% of the initial concentrations after 24 h and 96 h, respectively (Table 10).

Table 9

Time weighted average concentrations for measured TEH and total PAH in the Corexit CEWAF for the large volume toxicity tests

Species, life stage	Corexit % CEWAF	Corexit CEWAF TEH (mg/L) Time = 0	Corexit CEWAF TEH (mg/L) Time = 6	Corexit CEWAF TEH (mg/L) Time = 24	Corexit CEWAF TEH (mg/L) Time = 96	Time weighted average Concentration (mg/L)
Fish, adult	0.62	9.90	0.00	4.20	0.00	0.87
	1.85	27.3	17.7	13.7	4.10	6.74
	5.56	154	71.8	49.6	18.6	28.0
	16.7	397	256	212	69.6	108
	50	850	1126	NM	NM	
	100	2892	NM	NM	NM	
Shrimp, adult	0.62	3.70	3.60	4.40	3.40	3.60
	1.85	18.1	13.1	15.40	2.80	5.79
	5.56	67.6	70.3	52.80	7.60	19.6
	16.7	216	122	51.40	27.3	37.8
	50	550	548	NM	NM	
	100	1150	818	NM	NM	
Species, life stage	Corexit % CEWAF	Corexit CEWAF Total PAH (mg/L) Time = 0	Corexit CEWAF Total PAH (mg/L) Time = 6	Corexit CEWAF Total PAH (mg/L) Time = 24	Corexit CEWAF Total PAH (mg/L) Time = 96	Time weighted average Concentration (mg/L)

Species, life stage	Corexit % CEWAF	Corexit CEWAF TEH (mg/L) Time = 0	Corexit CEWAF TEH (mg/L) Time = 6	Corexit CEWAF TEH (mg/L) Time = 24	Corexit CEWAF TEH (mg/L) Time = 96	Time weighted average Concentration (mg/L)
Fish, adult	0.62	0.18	0.06	0.03	0.01	0.02
	1.85	0.51	0.36	0.14	0.06	0.09
	5.56	1.04	1.02	1.00	0.25	0.43
	16.7	5.48	4.87	1.86	1.52	1.77
	50	11.6	30.28	NM	NM	
	100	20.2	NM	NM	NM	
Shrimp, adult	0.62	0.17	0.07	0.04	0.01	0.02
	1.85	0.49	0.23	0.20	0.07	0.10
	5.56	1.17	0.79	0.46	0.24	0.32
	16.7	2.61	1.96	1.74	0.53	0.84
	50	7.79	6.43	NM	NM	
	100	16.6	13.3	NM	NM	
<i>NM</i> not measured						

Table 10

Time weighted average concentrations for measured TEH and total PAH in the Finasol CEWAF for the large volume toxicity tests

Species, life stage	Finasol % CEWAF	Finasol CEWAF TEH (mg/L) Time = 0	Finasol CEWAF TEH (mg/L) Time = 6	Finasol CEWAF TEH (mg/L) Time = 24	Finasol CEWAF TEH (mg/L) Time = 96	Time weighted average Concentration (mg/L)
Fish, adult	0.62	0.00	0.00	0.00	0.00	0.00
	1.85	0.00	0.00	0.00	0.00	0.00
	5.56	2.10	0.00	0.00	0.00	0.02
	16.7	7.80	5.80	0.00	0.00	0.34

Species, life stage	Finasol % CEWAF	Finasol CEWAF TEH (mg/L) Time = 0	Finasol CEWAF TEH (mg/L) Time = 6	Finasol CEWAF TEH (mg/L) Time = 24	Finasol CEWAF TEH (mg/L) Time = 96	Time weighted average Concentration (mg/L)
	50	21.0	15.4	0.00	0.00	0.89
	100	37.6	NM	NM	NM	
Shrimp, adult	0.62	0.00	0.00	0.00	0.00	0.00
	1.85	0.00	0.00	0.00	0.00	0.00
	5.56	0.00	2.30	0.00	0.00	0.11
	16.7	7.50	8.50	4.60	0.00	1.33
	50	21.7	22.3	9.40	0.00	3.00
	100	37.0	39.6	23.7	0.00	6.64
Species, life stage	Finasol % CEWAF	Finasol CEWAF Total PAH (mg/L) Time = 0	Finasol CEWAF Total PAH (mg/L) Time = 6	Finasol CEWAF Total PAH (mg/L) Time = 24	Finasol CEWAF Total PAH (mg/L) Time = 96	Time weighted average Concentration (mg/L)
Fish, adult	0.62	0.00	0.00	0.00	0.00	0.00
	1.85	0.00	0.00	0.00	0.00	0.00
	5.56	0.04	0.01	0.00	0.00	0.00
	16.7	0.12	0.05	0.01	0.00	0.01
	50	0.45	0.16	0.03	0.03	0.04
	100	0.94	NM	NM	NM	
Shrimp, adult	0.62	0.01	0.01	0.00	0.00	0.00
	1.85	0.04	0.02	0.01	0.00	0.00
	5.56	0.13	0.09	0.03	0.00	0.01
	16.7	0.36	0.26	0.06	0.01	0.03
	50	1.58	0.81	0.18	0.03	0.11
	100	1.63	1.38	0.39	0.07	0.20

Species, life stage	Finasol % CEWAF	Finasol CEWAF TEH (mg/L) Time = 0	Finasol CEWAF TEH (mg/L) Time = 6	Finasol CEWAF TEH (mg/L) Time = 24	Finasol CEWAF TEH (mg/L) Time = 96	Time weighted average Concentration (mg/L)
NM not measured						

The time weighted average (TWA) hydrocarbon concentrations determined for the Corexit-CEWAFs were approximately 28% of the concentrations measured at the start of the experiments (Table 9), whereas the TWA concentrations for the Finasol-CEWAFs were approximately 9% of the initial measured concentrations (Table 10). A comparison of 96 h LC₅₀ values determined using initial concentrations versus TWA concentrations for the large volume toxicity tests shows that the TWA calculated LC₅₀ values for Corexit-CEWAF were approximately 64% lower than the LC₅₀ values calculated using the initial concentrations (Table 11). The TWA calculated LC₅₀ values for Finasol-CEWAF were approximately 92% lower than the LC₅₀ values calculated using the initial concentrations. Calculations of LC₅₀ values using the initial measured concentrations are most likely an underestimation of toxicity, because they do not account for chemical loss over the 96-h exposure.

Table 11

Comparison of 96 h LC₅₀ values (95% confidence interval) determined using initial concentrations ($t = 0$) versus time weighted average concentrations for the large volume toxicity tests

Measured conc.	Adult fish	Adult shrimp
	96 h LC ₅₀ (95% CI)	96 h LC ₅₀ (95% CI)
Corexit-CEWAF in mg/L		
TEH $t = 0$	515.56 (413.69–637.34)	105.40 (85.07–131.96)
TEH TWA	217.78 (ND)	22.34 (19.07–26.41)
Total PAH $t = 0$	6.96 (5.55–8.62)	1.44 (1.21–1.76)
Total PAH TWA	3.55 (ND)	0.44 (0.36–0.54)
Finasol-CEWAF Exposure in mg/L		
TEH $t = 0$	28.21 (24.49–32.25)	26.17 (23.18–28.97)
TEH TWA	0.93 (ND)	3.74 (3.17–4.53)

Measured conc.	Adult fish	Adult shrimp
	96 h LC (95% CI)	96 h LC (95% CI)
Total PAH $t = 0$	0.66 (0.56–0.78)	1.25 (0.82–1.68)
Total PAH TWA	0.04 (ND)	0.12 (0.10–0.14)
ND not determined		

Discussion

The toxicity values available in the literature for Corexit 9500 are in agreement with those determined in this study. For example, Fuller et al. (2004) reported an LC₅₀ of 180 mg/L for *C. variegatus*, whereas this study reported 153 mg/L for the same species. The response of *C. variegatus* to Corexit also is similar to another estuarine fish (*Fundulus heteroclitus*), which had a 96-h LC₅₀ value of 84 mg/L (DeLorenzo et al. 2012). Aurand and Coelho (2005) reported a 96-h LC₅₀ value for Corexit with larval (4 days old) *C. variegatus* of 182 mg/L, similar to the value determined for the *C. variegatus* early life-stage test in this study of 172 mg/L. A 96-h Corexit LC₅₀ value reported for mysids in the literature of 42.0 mg/L (Hemmer et al. 2011) was comparable to 71.61 mg/L reported in this study (32.8 mg/L).

Few ecotoxicity values were available for the oil dispersant Finasol OSR52 before this study. A 48-h LC₅₀ value of 9.37 mg/L was previously determined for *A. bahia* and a 96-h LC₅₀ of 11.66 mg/L for *Menidia beryllina* (USEPA 2003). A 48-h LC₅₀ of 24.95 mg/L Finasol was determined for *A. bahia* in this study. The LC₅₀ values determined for Finasol with the estuarine species tested in this study ranged from 4.06 to 177.56 mg/L. The acute toxicity of Finasol was generally three to five times that of Corexit for the estuarine species tested.

The most sensitive species tested with both Corexit and Finasol based on acute mortality was the larval life stage of the mud snail, *I. obsoleta*. Larval life stages were generally more sensitive than adult life stages. Embryos were comparatively insensitive to dispersants, which is consistent with previous findings of low permeability of the embryonic coat to other chemicals (DeLorenzo and De Leon 2010; DeLorenzo et al. 2006). Compared with environmental levels of oil dispersants reported by Kujawinski et al. (2011), which ranged from 10 to 100 µg/L

during and after the DWH event, the individual dispersant LC₅₀ values reported here are much higher (>3.81 mg/L).

When comparing the toxicity of the two dispersants prepared as dispersed-LSC oil CEWAFs, a different trend in toxicity was observed when using the nominal percent CEWAF to calculate LC₅₀ values, whereby the Corexit-CEWAF was significantly more toxic than the Finasol-CEWAF. The nominal toxicity values do not take into account differences in the amount of oil each dispersant delivered into the seawater. For example, in the adult fish exposure, the 100% Finasol CEWAF Total PAH concentration was 0.94 mg/L and TEH concentration was 38 mg/L, whereas the 100% Corexit CEWAF Total PAH concentration was 20.2 mg/L and TEH was 2892 mg/L. The greater bioavailability of oil would account for the greater toxicity seen in the Corexit-CEWAF compared with the same dilutions of Finasol-CEWAF. The LC₅₀ values determined for each CEWAF based on measured hydrocarbon concentrations further demonstrate greater toxicity in the Finasol-CEWAF than in the Corexit-CEWAF. For instance, the Finasol-CEWAF was 11–18 times more toxic than the Corexit-CEWAF to the early life stage of *C. variegatus*, based on measured TEH and PAH concentrations. The average hydrocarbon (TEH) concentration measured in the Finasol-CEWAF in this study (67 mg/L) is similar to that reported by Dussauze et al. (2014) of 46 mg/L TPH (total petroleum hydrocarbons). The differences between the relationship between Corexit and Finasol CEWAFs based on nominal and measured hydrocarbon concentrations are likely a function of Corexit being a more effective dispersant and Finasol being inherently more toxic; therefore, the toxicity of the CEWAFs is driven both by the hydrocarbon concentrations and that of the individual dispersants in a complex mixture.

Although the results from this study do not provide a direct comparison between dispersed and undispersed oil toxicity, we can generalize based on previously published studies. The toxicity of mechanically dispersed LSC oil (WAF) has been determined with several of the test species used in this study. Hemmer et al. (2011) reported a 48-h LC₅₀ of 2.7 mg/L TPH for *A. bahia*. Rossi and Anderson (1976) reported a 96-h LC₅₀ of 12.5 mg/L for *N. arenaceodentata*, and Anderson et al. (1974) reported 96-h LC₅₀ values of 200 mg/L for adult *P. pugio* and 29,000 mg/L TPH for adult *C. variegatus*. Compared with the dispersed oil LC₅₀ values determined using measured TEH concentrations in this study, dispersed oil would be less toxic to *A. bahia* (37.28 mg/L Corexit-CEWAF and 13.05 mg/L Finasol-CEWAF); less toxic to *N. arenaceodentata* based on Corexit-CEWAF (126.31 mg/L), but equally toxic based on Finasol-CEWAF (12.30 mg/L); more

toxic to *P. pugio* (105.40 mg/L Corexit-CEWAF and 26.17 mg/L Finasol-CEWAF); and more toxic to *C. variegatus* (515.56 mg/L Corexit-CEWAF and 28.21 mg/L Finasol-CEWAF).

Bejarano et al. (2014) compared available LC₅₀ data for various oils prepared as WAFs versus chemically dispersed with Corexit 9500 (CEWAF) and determined 78% of the CEWAF values were of lower or equal toxicity than WAF values, whereas 12% ranged from 1.55-fold to 8.09-fold greater toxicity. Differences in methods used to prepare WAFs and CEWAFs, particularly the mixing energy level, affect the amount of measured hydrocarbons in solution and thus may complicate comparisons between LC₅₀ values.

Several sublethal effects of dispersants alone were identified in this study, such as a reduction in embryonic hatching success in sheepshead minnows and grass shrimp, increased lipid peroxidation activity in grass shrimp, and acetylcholinesterase activity inhibition in mud snails. These results are consistent with previous studies of dispersants on other species. For example, embryonic hatching success of mallard ducks also was significantly impaired by Corexit (Wooten et al. 2011). Corexit 9500 also has been shown to cause oxidative stress, measured by an increase in lipid peroxidation activity, in a mammalian cell line (Zheng et al. 2014). Acetylcholinesterase inhibition has been seen with other surfactants similar to the surfactant components of Corexit and Finasol. For example, sodium dodecyl sulfate inhibited AChE in daphnia (Guilhermino et al. 2000) and exposure to sodium dodecylbenzenesulfonate inhibited AChE in the freshwater cladocera *Moina macrocopa* (Martinez-Tabche et al. 1997). These effects indicate potential for chronic effects due to reproductive or neurological impairment. In addition, energy to counter cellular membrane damage may come at the cost of reduced growth or fecundity. Energy is expended in detoxification processes, such as p450 enzyme induction. A previous study found that although LSC oil and a mixture of LSC oil and Corexit induced EROD activity in the mummichog, *Fundulus heteroclitus*, Corexit alone did not significantly induce EROD activity in the fish compared to seawater control (DeLorenzo et al. 2012). The results with *C. variegatus* in this study are consistent with that of *F. heteroclitus*, with no measured effect of either Corexit or Finasol alone on EROD activity, but when oil was present in the exposure (CEWAFs), there was a significant induction of *C. variegatus* EROD activity. There also was a significant decrease in lipid peroxidation activity in fish and snails exposed to dispersed oil, suggesting energy allocation to detoxification.

Overall, Corexit was identified as the more effective and less toxic dispersant. Finasol elicited greater toxicity in the individual dispersant trials and dispersed lower levels of hydrocarbons into seawater than Corexit in the CEWAF trials. The range of LC₅₀ values calculated for the estuarine organisms in this study based on TEH concentrations in the CEWAFs (Corexit-CEWAF of 10–1815 mg/L TEH and Finasol-CEWAF of 0.68–90 mg/L TEH) are relatable to TEH concentrations measured in the environment. For example, a mean of 202 mg/L TPH was reported for 66 DWH surface water samples (Sammarco et al. 2013). In the open ocean, the mechanical action of waves and immense water volume dissipate dispersant-formed droplets at a higher rate, but the closed, shallow nature of estuarine, tidal creek, and lagoonal habitats could prevent effective dissolution of dispersants. For this reason, and consistent with current spill response protocols, it is unlikely that dispersants would be applied in coastal or inshore waters. Each spill situation is unique, however, and the results of this study provide response managers with data to guide decisions specific to estuarine habitats. The results of our study demonstrate that dispersant toxicity is compound- and species-specific. Moreover, different dispersants elicit different chemical interactions with oil that will affect bioavailability and toxicity of oil compounds to aquatic species. Should a future oil spill require the use of dispersants, the results of this study will allow managers to make informed decisions regarding the use of Corexit® 9500 or Finasol® OSR 52, particularly when applied to Louisiana Sweet Crude oil.

Acknowledgements

The authors thank Debbie Payton, Scott Lundgren, Alan Mearns, and Gary Shigenaka of NOAA/National Ocean Service/Office of Response and Restoration–Emergency Response Division for helpful suggestions and scientific exchange of ideas during the proposal and experimental planning phase of this project. The authors thank Adriana Bejarano for assistance in acquiring the Corexit and oil for testing. Finasol was purchased from Total Fluides, Paris-La Defense, France. The authors also thank the student researchers who participated in this project: McCall Calvert, Jeffrey Good, Elaina Graham, Casey Hughes, Megan Meek, Mildred Thomas, and Alfre Wimberley. The authors thank Jeanine Morey, Dalina Viveros, and Maggie Broadwater for reviewing the manuscript. The scientific results and conclusions, as well as any opinions expressed herein, are those of the author(s) and do not necessarily reflect the views of NOAA or the Department of Commerce. The mention of any commercial product is not meant as an endorsement by the Agency or Department.

Electronic supplementary material

Below is the link to the electronic supplementary material.

Supplementary material 1 (DOCX 16 kb)

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