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Comparison of Acute Aquatic Effects of the Oil Dispersant Corexit 9500 with Those of Other Corexit Series Dispersants

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The acute aquatic toxicity of a new Corexit series dispersant, Corexit 9500, was evaluated and compared with that of others in the series using early life stages of two common nearshore marine organisms: the red abalone (*Haliotis rufescens*) and a kelp forest mysid (*Holmesimysis costata*). Spiked-concentration testing was performed under closed, flowthrough conditions, with dispersant concentrations measured in real time using UV spectrophotometry. Median-effect concentrations ranged from 12.8 to 19.7 initial ppm for *Haliotis* and from 158.0 to 245.4 initial ppm for *Holmesimysis.* The difference in sensitivity of the two types of tests was consistent with patterns seen with other oil dispersants. Also, these data indicate Corexit 9500 to be of similar toxicity to Corexit 9527 and 9554. Corexit 9500 represents a reformulation of a long-time industry ''standard,'' Corexit 9527, to allow use on higher viscosity oils and emulsions. The present data suggest that acute aquatic toxicity concerns surrounding the use of this newer dispersant should not be significantly different from those associated with the use of Corexit 9527.

INTRODUCTION

The decision-making process surrounding the use of oil dispersants is necessarily a cost–benefit analysis; both the acute toxicity and the efficacy of proposed dispersants must be assessed, and the consequences of both use and nonuse of dispersants must be weighed. When a significant amount of oil is spilled on water, the responders' task is to minimize damage to property and natural resources. Injury to mammalian and avian resources, although toxicologically complex, can be fairly easy to assess on the gross scale; oiled birds and mammals are relatively easy to find and count. However, the effects of oil and dispersants on subtidal and epipelagic organisms are more difficult to assess in the field. Therefore, even though they often represent less-than-ideal models of true environmental consequences, laboratory toxicity tests are used as a way of comparing the effects of different dispersants. By testing the different available agents using standardized models, it is possible to directly compare their relative effects.

For laboratory tests to be of value, they must be conducted using standardized and comparable methods. Indeed, the lack of standardization of methods in the field of oil and dispersant toxicity testing has led to an unfortunate lack of comparability of data sets, and a lack of coherent conclusions, even after more than two decades of research (National Research Council, 1989; Markarian *et al.*, 1995). Over the past several years, an exposure system has been developed that allows the testing of complex, volatile mixtures using microscopic organisms under precisely controlled, flowthrough conditions (Singer *et al.*, 1990, 1993). Standardized procedures have also been developed for testing dispersants, and oils, in a modeled-exposure regime that provides useful insight into effects that might be expected in the field (Singer *et al.*, 1991). It has been found that in field situations, when sea and wind conditions are sufficient for use of dispersants, concentrations generally fall below detection in a relatively short time (Raj and Griffith, 1979; Mackay and Wells, 1983; Bocard *et al.*, 1984). Therefore, the exposure regime developed involves the dilution of initial dispersant solutions at a rate sufficient to reach concentrations below analytical detection in <8 hr (Singer *et al.*, 1991).

Dispersants are complex mixtures, primarily containing both charged and uncharged surfactants, as well as solvents. Their purpose is to orient at the oil–water interface, lower interfacial tension, and thus facilitate the formation of small (<100 μm) mixed oil–surfactant micelles (Canevari, 1973, 1978; National Research Council, 1989). The acute toxicity of dispersants is generally attributed to the effects of their surface-active components on biological membranes; the typical reaction to surfactant exposure involves disruption of respiratory cells, often resulting from electrolytic and/or osmotic imbalance (Abel, 1974; Abel and Skidmore, 1975; McKeown and March, 1978; Wells, 1984; National Research Council, 1989).

The objectives of this investigation were (1) to evaluate the acute effects of a new oil dispersant, Corexit 9500, on the early life stages of two marine species, the red abalone (*Haliotis rufescens*) and a kelp forest mysid (*Holmesimysis costata*), and (2) to compare the effects of 9500 with those of other members of the Corexit series of dispersants. The species selected are of both ecological and economic significance in California and

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have been widely used in the state for regulatory testing over the past several years (Anderson *et al.,* 1990; Singer *et al.,* 1990, 1991).

MATERIALS AND METHODS

Test Organisms

Haliotis embryos were obtained through spawning of in-house broodstock animals using the H_2O_2 method of spawning induction (Ebert and Houk, 1984). Embryos were introduced into test chambers within 1 hr of fertilization, to ensure initial exposure before the first cell division (Hunt and Anderson, 1989; Anderson *et al.,* 1990).

Juvenile *Holmesimysis* were obtained from gravid wild females collected from kelp forest canopies near Monterey, California (Martin *et al.,* 1989; Anderson *et al.,* 1990). Upon release from the females' marsupium, juveniles were isolated by daily cohort and reared for 3 days prior to testing on newly hatched (<24-hr-old) *Artemia* nauplii (Argentemia Gold Label, Argent, Redmond, WA).

Test Chemicals

Toxicity testing involved the oil dispersant Corexit 9500, which is a recent addition to the Corexit series of oil spill cleanup agents (obtained *gratis* from Nalco/Exxon Energy Chemicals, L.P., Sugar Land, TX). Unfortunately, because of the proprietary nature of this product's formulation, precise identification of its constituents is not possible here. It is characterized by the manufacturer as containing a surfactant mixture similar to that of Corexit 9527, but with a more oleophilic solvent system intended to better accommodate penetration of higher viscosity oils and emulsions (Nalco/Exxon, 1995). The surfactant profile of Corexit 9527 consists of both anionic and nonionic constituents, including oxygenated sorbitan mono- and trioleates, sorbitan monooleate, and sodium dioctyl sulfosuccinate. The more oleophilic solvent in Corexit 9500 is characterized only as a ''glycol ether, carboxylic acid salt.'' This dispersant was found to be soluble in seawater to 1000 ppm (v/v), which was well above working test concentrations.

Exposure System

A closed, flowthrough exposure system was employed in these tests. The various components of the system have been described previously (Singer *et al.,* 1990, 1991, 1993). The system utilizes sealed glass exposure chambers (≈260 ml) with integral fritted glass disks for containing microscopic test organisms in flowthrough conditions (Singer *et al.,* 1993). These test chambers are sealed by means of a two-part glass flange and O-ring held together with a full-circumference clamp (Fig. 1). Because sealed conditions can be maintained throughout toxicity tests, solution chemistry can be rigidly controlled, which is especially important when testing complex volatile and/or unstable mixtures. In order to monitor water quality within the test yet still maintain tight chemical control, 125-ml sampling flasks are located downstream of one chamber in each test treatment (Singer *et al.,* 1990). Because these flasks directly drain the test chambers, it is possible to characterize, in real time, the water quality conditions that exist within the test without disturbing the chambers themselves. All chambers were maintained in a temperature-controlled water bath and were supplied with a constant flow of diluent via a unified-drive multichannel peristaltic pump (Ismatec Model 7223, Cole–Parmer, Chicago, IL) fitted with 18 pump head cartridges, 1 for each chamber.

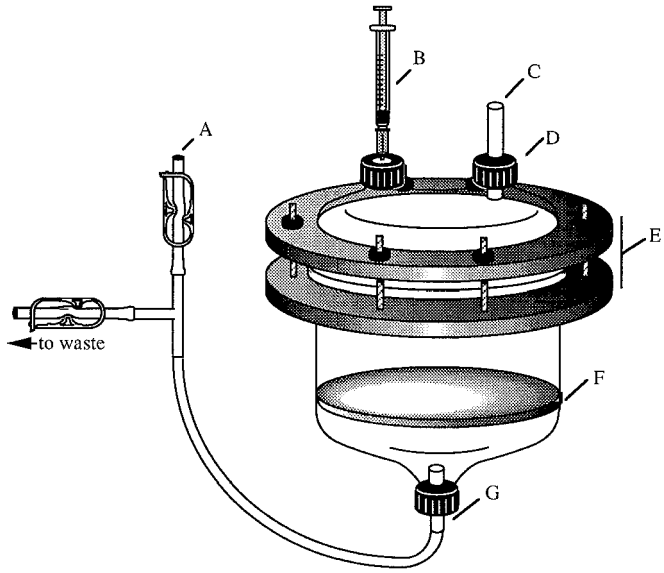

FIG. 1. Schematic of the toxicity test exposure chamber: (A) chemistry sampling arm; (B) syringe for food introduction; (C) diluent inlet; (D) threaded glass fittings with phenolic caps; (E) silicone O-ring-sealed glass flange with clamp; (F) fritted glass disk; (G) discharge outlet.

The overall system configuration began with a 4-liter, constant-volume, aerated head tank of seawater that fed each pump head cartridge individually (Fig. 2). Each cartridge then fed an exposure chamber directly. Having all cartridges on a single pump drive eliminated the potential for exposure variation associated with differential drift that might occur with multiple pumps. Exposure chambers were arranged in three rows of six (18 total), representing $n = 3$ replication in each of the six treatments. Two of the three replicate chambers in each treatment drained directly to waste, whereas the third drained to a sampling flask before going to waste. Each chamber's drain line was fitted with an accessory arm from which chemistry samples were collected in real time using the system's flow to fill the sampling pipette.

Test Procedures

Short-term, acute test procedures were used throughout this investigation: *Haliotis* tests were 48 hr in duration and involved the sublethal endpoint of larval shell deformation, whereas *Holmesimysis* tests lasted 96 hr and had a lethal endpoint (Anderson *et al.,* 1990). Water temperature, dissolved oxygen concentration, and pH were monitored daily during

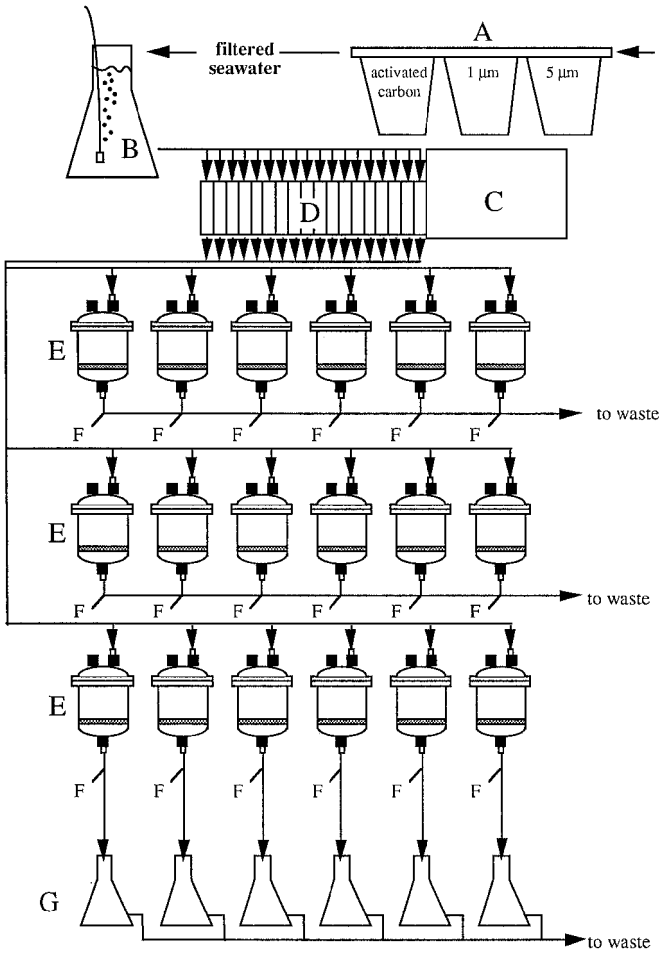

FIG. 2. Exposure system schematic exhibiting flow patterns and main system components: (A) cartridge filters; (B) seawater head tank; (C) peristaltic delivery pump; (D) cartridge pump heads (18); (E) exposure chambers; (F) chemistry sampling arms; (G) water quality sampling flasks.

testing. Diluent was natural seawater filtered to 1 μm (sand, cotton, and activated carbon filtration) at ambient salinity (≈33‰). Spiked-exposure test protocols followed those previously reported, with each test consisting of five toxicant treatments and a diluent control, each of which had three replicates (Singer *et al.*, 1991, 1993). Each dispersant concentration was prepared separately on a v/v basis, rather than serially diluted from a common stock, to ensure accuracy and repeatability.

Spiked exposures were accomplished by first prefilling test chambers with the appropriate dispersant concentrations. After temperature equilibration, test animals were loaded into chambers in random order at the appropriate density (≈1000 *Haliotis* embryos, or 8 juvenile *Holmesimysis*, per chamber). The test was then started by immediately initiating flushing with diluent following completion of organism addition; concentrations in each chamber were then measured hourly for 7 hr to document the decline profiles (Singer *et al.*, 1991).

All Corexit 9500 concentration measurements were made with a Perkin–Elmer Lambda 2 UV/VIS spectrophotometer (Norwalk, CT). Spectral analysis indicated it to absorb well in the UV range, with a functional λ_{max} of 234 nm (Fig. 3).

After 48 hr, all *Haliotis* larvae were fixed in buffered formalin and test endpoints were evaluated by microscopic examination (100×) of 100 haphazardly selected larvae for shell deformation or other gross abnormalities. *Holmesimysis* mortality was recorded daily, coincident with water quality measurement. *Holmesimysis* were provided food during testing at a rate of 40 *Artemia* nauplii per animal daily; total food amount was adjusted for the number of live animals at each feeding.

Statistical Analysis

Variation both within and among test populations was assessed by using three replicate exposure chambers within each test treatment and by running three replicate tests for each species. A NOEC was calculated for each test by means of a one-way ANOVA followed by Dunnett's (q') multiple-range comparison against a control (Zar, 1974). Median lethal concentrations (EC_{50}/LC_{50}) were estimated by the trimmed Spearman–Karber technique (Hamilton *et al.*, 1977). Reproducibility of toxicity data was assessed using the CV of median-effect estimates for the triplicate tests (Schimmel *et al.*, 1989).

Test acceptability was determined using both biological and chemical data. Biological acceptability criteria followed those of established test protocols; generally, <20% effect in controls was deemed acceptable. Chemical acceptability criteria were established in earlier spiked-exposure testing (Singer *et al.*, 1991, 1993, 1994); similarity of chemical concentration decline rates both within and among tests was essential to establish that all tests modeled similar conditions. Linear regressions of log-transformed concentrations versus time for all treatments within a test were compared by analysis of covari-

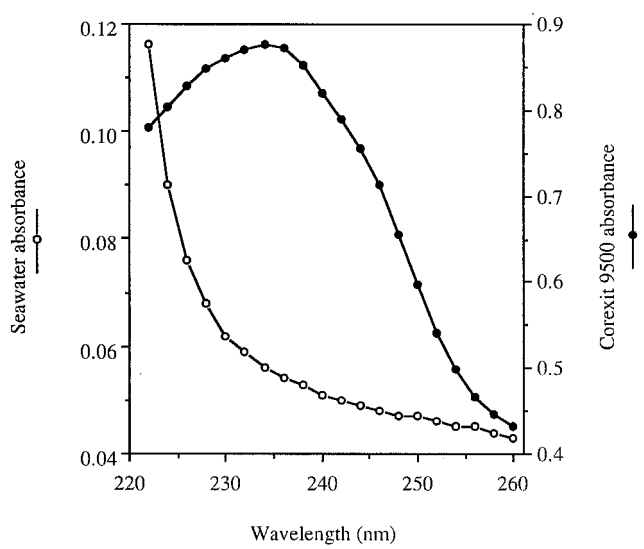

FIG. 3. Representative UV scans of 1-μm filtered seawater and a 250-ppm solution of Corexit 9500.

ance to determine that the concentration decline slopes for each treatment were not significantly different ($\alpha = 0.05$). If any treatment was found to be significantly different, the treatment was not used in endpoint estimation; if endpoint estimation was thus compromised (i.e., loss of two or more treatments), the entire test was considered unacceptable. Once within-test treatment equality was established, comparison among tests was performed by use of standard ANOVA with mean slope data.

RESULTS

Test Conditions

In all cases, test temperatures varied by <1°C, pH varied by <0.9 units, and oxygen concentrations were maintained at 76 to >100% saturation (Table 1).

All test concentrations were below dispersant solubility limits. Standard curves constructed for each test showed high linearity ($r^2 \geq 0.99$), and nominal and verified initial test concentrations had high concordance in all tests; *Haliotis* test verified concentrations were generally slightly higher than nominal (regression slope, 1.129; $r^2 = 0.996$), whereas verified *Holmesimysis* test concentrations were generally slightly less than nominal (slope, 0.938; $r^2 = 0.985$). All toxicity data presented here are based on spectrophotometrically verified initial concentrations (Singer *et al.*, 1991).

Dispersant concentrations generally declined to below detection limits within 6–8 hr. Comparison of first-order chemical decline rate constants (K_e) both within and among tests indicated no significant difference ($P > 0.05$). Rate constants ranged from 0.35 to 0.61 hr^{-1} (mean, 0.49) in *Haliotis* tests, and from 0.37 to 0.62 hr^{-1} (mean, 0.50) in *Holmesimysis* tests.

TABLE 1
Summary of Water Quality Parameters Monitored during Corexit 9500 Toxicity Testing

	Haliotis	*Holmesimysis*
Temperature (°C)		
Mean	15.05	13.42
SD	0.27	0.79
Range	14.6–15.4	11.7–14.2
Maximum single-test change	0.7	0.6
pH (units)		
Mean	7.88	7.73
SD	0.24	0.25
Range	7.35–8.40	7.24–8.14
Maximum single-test change	0.7	0.83
Dissolved oxygen (ppm)		
Mean	7.31	7.62
SD	0.49	0.36
Range	6.54–8.45	6.64–8.51
Maximum single-test change	1.07	1.44

Note. Pooled triplicate test data except where noted.

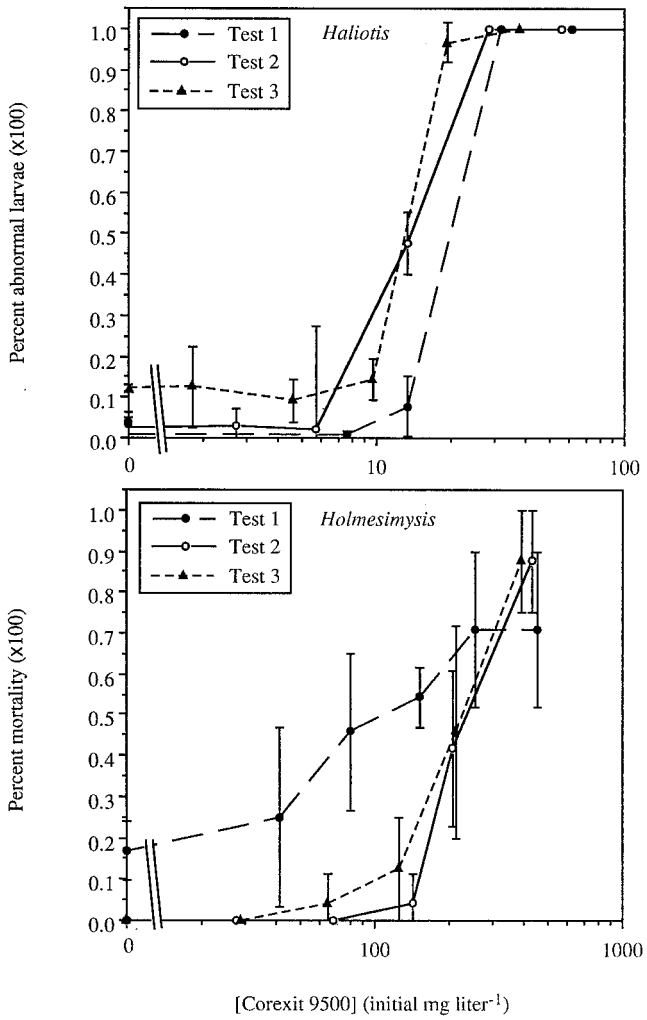

FIG. 4. Dose–response curves for triplicate *Haliotis* (top) and *Holmesimysis* (bottom) toxicity tests. Data symbols represent means ± SD for each treatment ($n = 3$).

Haliotis Tests

Haliotis tests were conducted with initial concentrations ranging from 2 to 100 ppm and demonstrated good dose–response relationships and reproducibility (Fig. 4; Table 2). NOEC estimates ranged from 5.7 to 9.7 initial ppm, while EC_{50}s ranged from 12.8 to 19.7 initial ppm. EC_{50} estimates had a CV of 24.8%, and inspection of 95% fiducial limits, which were fairly narrow, suggested that the three estimates were significantly different from each other.

Holmesimysis Tests

Holmesimysis test initial concentrations ranged from 25 to 500 ppm. The dose–response relationship of the first test was substantially different from those of the second and third tests, which were very similar (Fig. 4; Table 2). Toxicity estimates for the three tests reflect a similar intertest pattern, with both the NOEC and the LC_{50} of the first test being substantially

TABLE 2
Results of Triplicate Corexit 9500 Toxicity Tests Using *Haliotis* and *Holmesimysis*

Test		NOEC	EC_{50} (95% CL)
Haliotis	1	7.6	19.7 (19.5, 20.0)
	2	5.7	12.8 (12.4, 13.1)
	3	9.7	13.6 (13.4, 13.7)
Holmesimysis	1	41.4	158.0 (103.1, 242.0)
	2	142.3	245.4 (207.5, 290.1)
	3	124.4	223.7 (188.3, 265.7)

Note. All data expressed in initial ppm (v/v).

different from those of the other two. NOECs ranged from 41.4 to 142.3 initial ppm, with LC_{50}s ranging from 158.0 to 245.4 initial ppm. Despite this variability, the CV for LC_{50} estimates was only 21.8%.

A high proportion of delayed mortality was observed in all *Holmesimysis* tests (Table 3). Only about one-third of all recorded mortalities occurred during the first 72 hr of the first and third tests (32 and 31%, respectively), and during the first 48 hr of the second test (34%).

DISCUSSION

The present data have indicated the *Haliotis* test to be significantly more sensitive than the *Holmesimysis* test; this is consistent with all past results (Singer *et al.,* 1991, 1993, 1994, 1995). These differences in sensitivity are likely the result of life stage and/or test endpoint differences. Abalone tests utilized undeveloped embryos, exposed during critical early cell divisions, whereas mysid tests involved fully developed juveniles and used a coarser endpoint, mortality. It is therefore likely that the mode of toxic action in these tests is different (Singer *et al.,* 1993).

It was found that Corexit 9500 elicits effects similar to those seen with other dispersants (Singer *et al.,* 1991, 1993, 1995). In *Haliotis* tests, surface-active compounds in the dispersant likely affect the embryonic membrane. This is evidenced by the fact that developed, abnormal larvae were virtually nonexistent; during endpoint evaluation, observers either found fully developed, normal larvae or embryos that had been arrested at the multicell stage, often appearing as only loose aggregations of cells. These observations are consistent with known effects of surfactants on biomembranes, e.g., increased permeability, loss of barrier function, osmotic imbalance (Benoit *et al.,* 1987; Partearroyo *et al.,* 1990). Also, the occurrence of several pathological abnormalities in developing embryos has been reported in marine echinoderms and other gastropods (Tanaka, 1976; Render, 1990).

Some mysid mortality may ultimately be caused by similar membrane effects, but these effects would more likely manifest in the form of asphyxiation caused by damage to respiratory structures (Swedmark *et al.,* 1971; Abel, 1974, 1976). In past testing, delayed mortality seen in mysids suggested that these animals might possess protective structures, such as thick epithelial cuticles, that could limit exposure or were able to ameliorate biochemical toxicity through physiological pathways, such as biotransformation and depuration (Swedmark *et al.,* 1971). This is borne out by the observation that juvenile *Holmesimysis* have been seen to survive in constant dispersant exposures of up to 30 ppm for up to 48 hr before significant mortality occurred (Singer *et al.,* 1990). It appears, though, that the effects of Corexit 9500 in mysids may be more biochemical than those of the other Corexits, as evidenced by the relatively long lag time before the onset of significant mortality (Table 3), which may imply a greater solvent role in its toxicity. Initial contact with 9500 did not appear to elicit much of an immediate effect, but after several days of survival, mortalities occurred quite rapidly, suggesting the reaching of some physiological or biochemical threshold of damage that was slower to manifest than simple asphyxia.

According to its manufacturer, Corexit 9500 represents the reformulation of an existing agent and is meant to widen the opportunities for dispersant use on higher viscosity (weathered) oils and emulsions. The present data suggest that the acute effects of 9500 on marine animals are not significantly different from those of its predecessors, Corexit 9527 and 9554 (Fig. 5; Singer *et al.,* 1991, 1995). Comparison of median-effect concentrations among Corexit 9500, 9527, and 9554 demonstrated no significant difference in mysid tests, no difference between 9500 and 9527 in abalone tests, and that 9554 was significantly more toxic to abalone ($\alpha = 0.05$). Corexit 9500 testing resulted in lower repeatability of toxicity estimates (based on EC/LC_{50} CVs) than was seen in testing of either of the other dispersants. The reason for this is unknown; however, given the observation that these species produced

TABLE 3
Daily LC_{50} (95% Confidence Limits) Estimates for Triplicate Corexit 9500 Tests

	Observation time (hr)			
	24	48	72	96
Test 1	>453.4	>453.4	>453.4	158.0 (103.1, 242.0)
Test 2	>436.3	>436.3	279.6 (210.3, 371.7)	245.4 (207.5, 290.1)
Test 3	>392.1	>392.1	>392.1	223.7 (188.3, 265.7)

Note. When less than 50% mortality was observed, estimate is reported as ''greater than'' highest test concentration. All data are expressed in initial ppm (v/v).

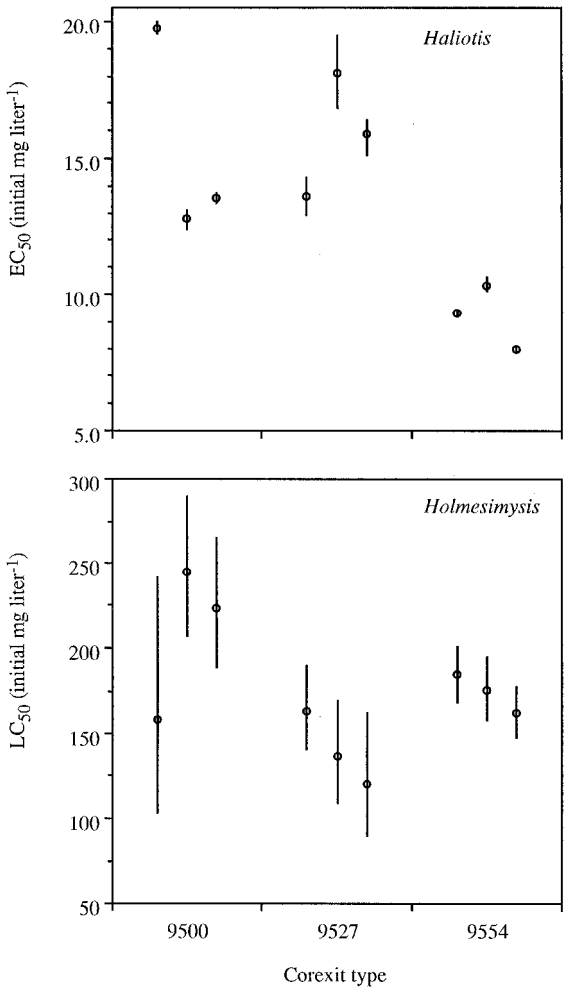

FIG. 5. Comparison of median-effect concentrations of triplicate *Haliotis* (top) and *Holmesimysis* (bottom) toxicity tests using Corexit 9500, 9527, and 9554. Data symbols represent EC/LC_{50} with 95% confidence intervals.

highly repeatable endpoint data with other toxicants during the same time period, test population variability does not appear to be a factor. Also, whereas final 96-hr mysid LC_{50}s were similar among all three agents, 9500 data indicated substantially more delayed mortality; the majority of test animals survived up to 2–3 days after initial exposure before dying (Fig. 6). This may be reflective of differences in the formulation of 9500, but without specific constituent information, which is proprietary and unavailable, no specific conclusions can be drawn. It is unlikely that differences in repeatability among agents were simply the result of natural variation in biological response, because these three agents were tested at widely different times (9527 in 1990, 9554 in 1993, and 9500 in 1995), yet 9500 toxicity estimates were consistently more variable in both species.

CONCLUSIONS

The acute effects on two marine species of the recently introduced oil dispersant Corexit 9500 were evaluated using

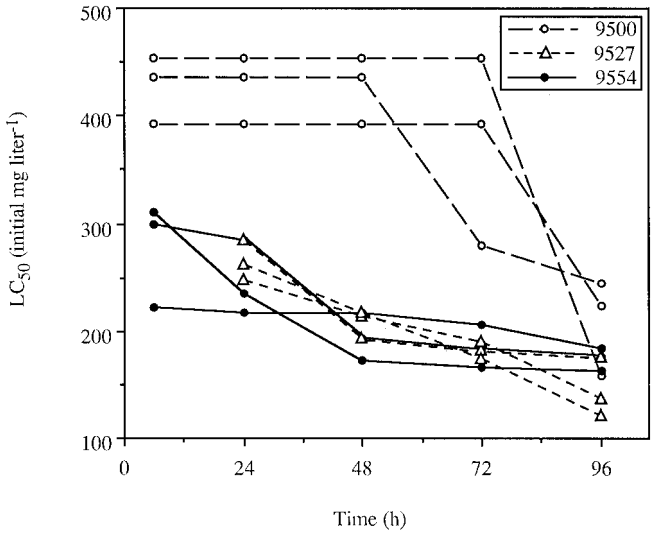

FIG. 6. Daily LC_{50}s for triplicate *Holmesimysis* toxicity tests using Corexit 9500, 9527, and 9554. For clarity, data are provided without 95% confidence intervals.

modeled-exposure, flowthrough toxicity tests. This dispersant was found to be highly soluble in seawater (up to 1000 ppm) and easily quantified directly by UV-VIS spectrophotometry. Corexit 9500 was seen to elicit acute effects at initial concentrations of 12–20 ppm in embryo/larval abalone tests and 150–245 ppm in juvenile mysid tests; the effects seen were typical of surfactant-based dispersants. Based on the results obtained, it was found that issues of direct dispersant toxicity surrounding the use of Corexit 9500 do not appear to be significantly different from those associated with the use of Corexit 9527.

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