TOXICITY OF PHYSICALLY AND CHEMICALLY DISPERSED OILS UNDER CONTINUOUS AND ENVIRONMENTALLY REALISTIC EXPOSURE CONDITIONS: APPLICABILITY TO DISPERSANT USE DECISIONS IN SPILL RESPONSE PLANNING

James R. Clark, Gail E. Bragin, Eric J. Febbo, and Daniel J. Letinski
ExxonMobil Biomedical Sciences, Inc.
Annandale, New Jersey 08801-0971

ABSTRACT: *As part of efforts to develop standardized testing protocols under the Chemical Response to Oil Spills Environmental Research Forum (CROSERF) and apply the results to real-world scenarios, three types of oils and two dispersants were tested in both continuous and short-term spiked exposures using the early life-stages of several marine organisms. Test species included embryo-larval stages of Pacific oyster (*Crassostrea gigas*), two marine mysids (*Holmesimysis costata *and* Mysidopsis bahia*), and two marine fishes (turbot,* Scophthalmus maximus *and inland silverside,* Menidia beryllina*). Oils were physically dispersed in seawater by vortex mixing in a flask and chemically dispersed using the same approach with COREXIT® 9527 or COREXIT® 9500 applied in a 10:1 oil-to-dispersant ratio to generate maximum exposure concentrations. Continuous exposure tests followed standard testing protocols for 96-hour or 48-hour duration, according to demands of the test species. Spiked exposures reflect continuous dilution of water column concentrations (half-life ~107 minutes), as observed in the field when oil is dispersed into open waters. Results are reported as the acute LC50s. Tests oils included fresh and weathered Kuwait crude, fresh Forties crude, and a Medium Fuel Oil (MFO) mix. Exposure concentrations for oil tests were quantified using gas chromatography and expressed as the sum of the C_{10} to C_{36} components, or $TPH_{(resolved)}$. Dispersant exposure concentrations were verified by UV spectrophotometric analysis. Not all species were tested with each oil and dispersant.*

For dispersants tested individually, constant exposure LC50s ranged from 3 to 75 mg/L, with oyster the most sensitive and turbot the least sensitive species. Spiked exposure LC50s ranged from 14 to >1055 mg/L among all test species. Dispersants were up to 36 times less toxic under spiked exposure conditions compared to similar treatments under constant exposure conditions. For oils, LC50s based on $TPH_{(resolved)}$ are similar for both the physically and chemically dispersed oil, demonstrating that dispersant did not increase the toxicity of oils based on measured exposures. Under constant exposure conditions, test species are very similar in sensitivity to the oils, with most LC50s around 0.5 ppm $TPH_{(resolved)}$. Spiked exposures were 4 to 100 fold less toxic to these test organisms. The more environmentally realistic spiked exposures demonstrate that standard, continuous exposure test data overestimate the potential toxicity of dispersed oil. When laboratory toxicity data are used as part of a dispersant approval process for spill response, the decision should take into account whether exposure durations and sensitivity of test species are representative of conditions in the spill area.

Introduction

In oil spill response, the purpose of using dispersant is to move the oil into the water column to disperse and dilute rapidly. If not effectively dispersed, oil on the water surface could come into direct contact with marine mammals and other wildlife; it will also continue to mix into the top meter of the water column and resurface for an extended duration. When effectively dispersed, there is a short-term exposure of water column organisms to the oil. If dispersants are not used, the wildlife populations face potentially prolonged exposures, and the potential for shoreline impacts increases. Since populations of water column organisms can recover much more rapidly than wildlife or shoreline communities, dispersing oil provides a potential net environmental benefit (Lewis and Aurand, 1997). Differences in exposure concentrations in the Water Accommodated Fractions (WAFs) of chemically and physically dispersed oil make use of toxic effects estimates based on oil loading rates an inappropriate basis for assessing exposure trade-offs, actual exposure concentrations must be measured to provide valuable comparisons. This is also true when loading rates are used to evaluate toxicity of WAFs generated by effective versus poor dispersants. Poor dispersants "appear" less toxic using this exposure basis and so may be accepted for use, when in actuality they do not disperse as much of the oil into the water column.

Environmental toxicity considerations typically are part of dispersant approval decisions around the world. Potential for acute toxicity of dispersants and dispersed oil to aquatic invertebrates, and fish are considered using data from a variety of standardized tests (Pauwels and Clark, 1993). Although such tests allow various products to be ranked by toxicity relative to each other, the tests do not address environmentally realistic, dynamic exposure conditions likely to occur during an actual oil spill. Decisions not to use dispersants based on these non-representative data may prohibit the use of a response action which is actually environmentally safe.

The use of standard laboratory test data to assess potential field effects is further complicated, as chemical dispersants change the rates at which oil and its constituents enter the water column. The results of laboratory toxicity studies thereby become "confusing" since determination of specific factors and constituents causing

toxic effects can be related to a number of physical and chemical factors (Singer *et al.*, 2001a, in press).

For these reasons and others, the Chemical Response to Oil Spills Environmental Research Forum (CROSERF) testing protocol was developed to evaluate the aquatic toxicity of WAFs generated by physically and chemically dispersing crude oil (Singer *et al.*, 2001a, in press). The tests reported here were conducted under the general CROSERF testing protocol, but with slight variations in techniques used to generate the WAFs. Specifically, the authors used mixing energy sufficient to generate a vortex in the mixing vessels to prepare both physically and chemically dispersed WAFs. The role of mixing energy was then evaluated as a factor in determining the nature of the constituents comprising exposures in the toxicity tests conducted under CROSERF.

Quantitative analytical chemistry techniques characterized the hydrocarbon components of the WAF generated in the authors' studies to quantify exposures, and to evaluate which hydrocarbons best explain the observed toxicity. A second objective was to compare the toxicity of these oils tested under standard, constant exposure conditions with toxicity observed under more environmentally realistic spiked exposure. The spiked exposures were designed to simulate a short-term, declining exposure representative of exposure regimes modeled and measured under physically and chemically dispersed oil slicks in offshore waters. A final consideration in assessing the relevancy of laboratory toxicity data was to compare lethal effect concentrations based on measured exposures with exposures based on the loading concept, which is common to many governmental dispersant approval programs outside of North America (Pauwels and Clark, 1993).

Materials and methods

General test method guidelines. Constant and pulsed exposure, 48-hour static tests with oysters were conducted in accordance with standard test methods (ASTM, 1994). Constant exposure, 48-hour static tests with turbot generally followed European (PARCOM, 1994) and U.S. methods (Weber, 1993). Constant exposure, static with daily renewal, 96-hour acute toxicity tests with mysids and silversides were conducted in accordance with U.S. Environmental Protection Agency methods (EPA, 1994; Weber, 1993). Continuous-flow toxicity tests were conducted according to the methods of Singer *et al.* (1990, 1993), and generally followed water quality and monitoring procedures specified in EPA toxicity test protocols (EPA, 1987; Weber, 1993). Several sources report details of the specific test procedures used to control exposures during pulsed and spiked exposures (Bragin and Clark, 1995; Bragin *et al.*, 1994; Coelho *et al.*, 1995; Pace and Clark, 1993; Singer *et al.*, 2001a).

Test organisms and materials. Commercially supplied or laboratory-reared test organisms were acclimated or cultured using techniques consistent with standard test methods and specific studies cited above. Tests were conducted with Kuwait crude oil obtained off of the lighter *Stena Concertia* in April 1994, Forties crude oil obtained from the *Forth Explorer* research vessel in 1995, and a medium fuel/gas oil (MFO) mixture obtained from AEA Technology Environmental in Abingdon, United Kingdom in 1994. Exxon Chemicals supplied dispersants used in the tests. COREXIT® 9527 was used in chemically dispersed oil tests with Kuwait crude and MFO; COREXIT® 9500 was used with Forties crude.

WAFs were prepared individually for each test concentration since the loading rate affects the solubility of oil in water (Singer *et al.*, in press). In contrast with the CROSERF procedure (Singer *et al.*, 2001a), both oil and chemically dispersed oil WAFs were prepared with sufficient mixing energy to form a 20 to 25% vortex depth in the mixing vessel. For chemically dispersed oil treatments, dispersant was added after the vortex was set. Dispersants were added at a 1:10 ratio to the volume of test oil. Methods for preparation of dispersant only test solutions are those reported by Pace and Clark (1993).

Analytical methods. Three main classes of hydrocarbon constituents served as the authors' target analyte list: resolved Total Petroleum Hydrocarbons ($TPH_{(resolved)}$), semi-volatiles (Polynuclear Aromatic Hydrocarbons, or PAHs), and volatiles. Gas chromatography with flame ionization detection was used for TPH analysis. Semi-volatile and volatile analyses followed standard EPA methods for these constituents. Analytical approaches are discussed in detail in Singer *et al.* (2001a).

Gas chromatography analyzed WAFs for resolvable petroleum hydrocarbons with chain lengths of C_{10} through C_{36} and reported as $TPH_{(resolved)}$. While these data do not represent, in actuality, total hydrocarbon content of a sample, this TPH method focuses on those constituents likely to be taken up by test organisms and cause acute, toxic effects. Subsamples of test concentrations bracketing LC50s were sampled for volatile and semi-volatile components. Details of the analytical chemistry are reported in Bragin and Clark (1995) and Bragin *et al.* (1994).

Dispersant-only exposure concentrations were verified using a Hewlett-Packard (Model 8450A or 8543) single beam UV-VIS spectrophotometer. The Model 8543 spectrophotometer was controlled with a Hewlett Packard Vectra VL Computer equipped with HP ChemStation software. All runs were made with a 10-mm light path cuvette and scanned from 225 to 235 nm (Model 8450A), or 200 to 300 nm (Model 8543) with absorbance measurement taken at 229 or 230 nm. Calibration curves consisting of five to eight standards were generated each day of analysis with the high and low standards bracketing the concentration range being tested. All standards were made in seawater. An Ion Chromatographic (IC) technique was used for concentration verification for tests performed prior to 1994 (flow-through, constant exposure tests) (Pace and Clark, 1993).

All tests were conducted in an environmental chamber that continuously monitors and controls temperature and light intensity. In addition, test water temperature was monitored throughout the tests at each treatment level.

Results and discussion

General water quality parameters (temperature, dissolved oxygen, pH, and salinity) remained within the required range during the tests, with a few minor exceptions that would not significantly impact the test results. Median lethal effect concentrations (LC50) and median lethal loading levels (LL50) were calculated using either probit transformations (Finney, 1971), or the Spearman-Karber test (Hamilton *et al*., 1977). In a few instances, data were smoothed and/or corrected for control mortality following procedures outlined in EPA guidelines (Weber, 1993). Results of toxicity tests and LC50 calculations are summarized in Tables 1 and 2.

Toxic constituents in physically and chemically dispersed oils. Figure 1 shows the relationship between mortality and exposure concentrations for each of the major chemical constituent classes ($TPH_{(resolved)}$, PAHs, Volatile Organic Analyses or VOAs) and loading level (Load). The figures contain data for both Kuwait and Forties crude oil tests with *M. bahia*

Table 1. LC50s for constant and spiked exposure toxicity tests based on measured concentrations of oil, dispersed oil, or dispersant for five test species.

		Constant exposure		Spiked exposure	
Organism	**Oil / dispersant**	**LC50 (mg/L)**	**95% CI**	**LC50 (mg/L)**	**95% CI**
Crassostrea gigas (oyster)	Kuwait + C9527	0.50	0.05–1.74	1.92	0.94–6.16
	Forties + C9500	0.81	0.52–1.39	3.99	3.07–5.45
	Medium Fuel Oil	>1.14		>1.83	
	MFO + C9527	0.53	cnc	2.28	1.69–3.35
	COREXIT® 9527	3.09	3.06–3.12	13.9	9.17–31.1
Holmesimysis costata (kelp mysid)	Kuwait	0.10	0.08–0.13	>2.76	
	Kuwait + C9527	0.17	cnc	1.80*	1.17–3.51
	COREXIT® 9527	9.74	7.39–13.8	195	135–282
Mysidopsis bahia (gulf mysid)	Kuwait	0.63	cnc	>2.93	
	Kuwait + C9527	0.65	0.52–0.82	17.2†	11.3–28.8
	Weathered Kuwait			>0.17	
	Weathered Kuwait + C9527	0.11	0.09–0.14	111	80.4–158
	Forties + C9500	0.42	0.34–0.52	15.3	13.6–17.9
	COREXIT® 9527	29.2	26.4–32.3	>1014	
	COREXIT® 9500	35.9	32.2–41.3	>789	
Scophthalmus maximus (turbot)	Kuwait + C9527	2.00	1.74–2.31	16.5	cnc
	Forties	0.35	cnc	>1.33	
	Forties + C9500	0.44	0.39–0.49	48.6	35.9–109
	COREXIT® 9500	74.7	57.6–101	>1055	
Menidia beryllina (silversides)	Kuwait	0.97	0.83–1.29	>1.32	
	Kuwait + C9527	0.55	0.41–0.74	6.45	3.94–10.7
	Weathered Kuwait	0.14‡	cnc	>0.66	
	Weathered Kuwait + C9527	1.09	0.96–1.28	10.9	9.89–12.0
	Forties + C9500	0.49	0.40–0.59	9.05	7.70–10.2
	COREXIT® 9527	52.3	47.9–57.1	58.3	55.6–61.1

Note: Oil data reported as $TPH_{(resolved)}$, dispersants quantified by spectrophometry.
* Pooled data from three tests: (1) 5.04 (3.64–8.08); (2) 1.73 (1.40–2.33); (3) 1.30 (0.02–1.66).
† Pooled data from four tests: (1) 13.2 (10.5–16.2); (2) 24.8 (16.0–75.6); (3) 23.8 (cnc); (4) 17.1 (cnc).
‡ Value is extrapolated.

and *M. beryllina* since these organisms responded similarly under the test conditions. $TPH_{(resolved)}$ demonstrates the most significant dose-response relationship, with R^2 values of ~0.6 to 0.7. Neither PAHs nor volatiles correlate concentration to toxicity, with R^2 values <0.2. In addition, at higher concentrations (used in the spiked exposure tests) percent mortality does not increase with increasing concentration for either the volatile fraction or oil loading.

Since WAFs for both physically and chemically dispersed oils were prepared with mixing energy sufficient to develop a vortex, water samples from physically dispersed WAFs contained considerably more TPH compared to WAFs from other CROSERF tests, where the oil-only WAF was prepared from a gently stirred flask. In those results, the volatile fraction makes up a greater percentage of the exposure media for physically dispersed oil WAFs (Singer *et al.*, 2001a). Even in the absence of dispersants, the mixing that the authors used to prepare WAFs generated a few small droplets of entrained oil, contributing significantly to the authors' TPH values. These exposures provide a more realistic comparison of what might be encountered under an oil slick with wave action in open waters, even though the authors' data are likely to be inherently more variable because of the complications from entraining oil droplets in the vortex during mixing.

Loading as a measure of exposure. There was some relationship between TPH and loading when individual oils and chemically dispersed oils were evaluated separately (Figure 2). Tested oils entered the water column at different rates over the range of loading levels tested. The correlation between loading and $TPH_{(resolved)}$ is strong, with R^2 values ranging from 0.86 to 0.92 for WAFs prepared using physical dispersion techniques. Significantly more oil (or TPH) enters the water at a given loading level when dispersant is used. The correlation remains strong, with R^2 values ranging from 0.81 to 0.90 for chemically dispersed oil. Over a range of loadings, physical dispersion of crude oil resulted in measured $TPH_{(resolved)}$ values that represented approximately 0.08% of the total oil introduced into the test system. In contrast, dispersant use resulted in measured $TPH_{(resolved)}$ values approximately 2.2% of the initial oil loading rate. These data agree with Lunel (1994) who reported water column concentrations between 0.1–0.2% and 1.8–3.5% of the oil initially released during sea dispersant trials under control (physically dispersed) and chemically dispersed slicks, respectively.

Table 2. Lethal loading results: Data does not correlate with toxicity.

		Constant exposure		Spiked exposure	
Organism	**Oil / dispersant**	**LL50 (mg/L)**	**95% CI**	**LL50 (mg/L)**	**95% CI**
Crassostrea gigas (oyster)	Kuwait + C9527	29.7	5.55–95.5	72.9	44.0–158
	Forties + C9500	34.4	17.6–90.9	96.4	58.4–227
	Medium Fuel Oil	>1108		>26256	
	MFO + C9527	23.0	11.2–124	59.1	32.2–448
Holmesimysis costata (kelp mysid)	Kuwait	39.5	27.4–54.1	>24323	
	Kuwait + C9527	27.4	23.4–32.7	220	156–335
Mysidopsis bahia (gulf mysid)	Kuwait	618	280–981	>24323	
	Kuwait + C9527	92.9	78.3–110	1083	682–1883
	Weathered Kuwait	184	67.8–573	>25691	
	Weathered Kuwait + C9527	32.6	25.5–40.9	16782	11266–20033
	Forties + C9500	27.1	cnc	2192	cnc
Scophthalmus maximus (turbot)	Kuwait + C9527	138	101–198	1432	904–2917
	Forties	156	137–176	>23471	
	Forties + C9500	36.2	27.6–49.1	1612	1382–2025
Menidia beryllina (silverside)	Kuwait	5020	4208–6131	>25000	
	Kuwait + C9527	112	97.7–128	864	698–1114
	Weathered Kuwait	2319*	1688–4058	>26879	
	Weathered Kuwait + C9527	381	332–454	2418	2152–2717
	Forties + C9500	37.3	33.2–41.0	252	208–304

* Value is extrapolated.

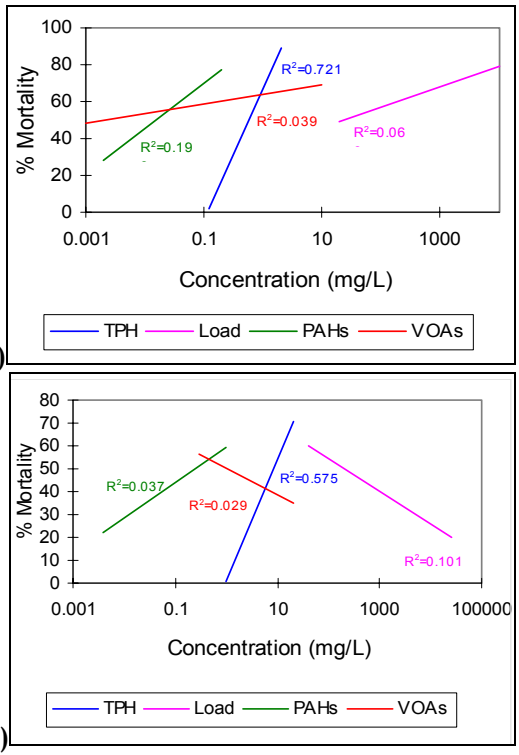

Figure 1. Relationships between mortality and various measures of exposure. (a) Constant exposure tests. (b) Spiked exposure tests. TPH, Total Petroleum Hydrocarbons, C10–C36; Load, amount of oil loaded into vessels for WAF preparation; PAHs, Polynuclear Aromatic Hydrocarbons; VOAs, constituents from Volatile Organic Analyses. See methods section for methodological references.

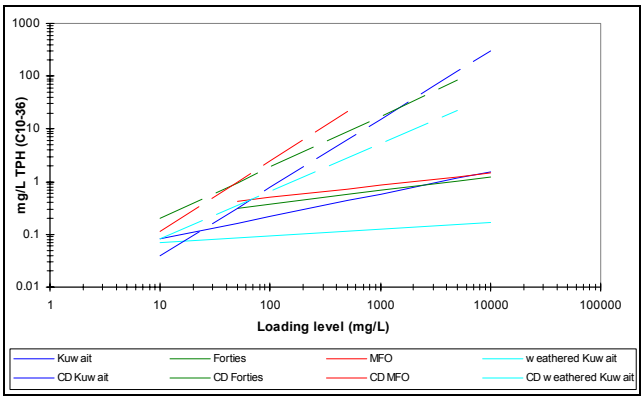

Figure 2. Relationship between measured $TPH_{(resolved)}$ for various oil loading levels for WAFs prepared using physical dispersion (oil plots) or chemical dispersion (CD Oils).

Although TPH concentration in a WAF and loading can be related for physically or chemically dispersed oil, the toxicological responses of test organisms are best explained by examining actual exposure concentrations in the WAF. Further, toxicity of oil and chemically dispersed oil can not be directly compared using the lethal loading concept. Dispersant changes the exposure factor for oil (the amount of TPH in water per unit oil). The authors observed that 25 to 30 times more oil enters the water column when dispersants are used with lower loading rates for the oils tested. This is reflected in the disparities in lethal effect concentrations when comparing LL50s and LC50s in Table 2. Based on loading, physically dispersed oil appears less toxic than chemically dispersed oil. However, similar dose-response relationships are seen for physically and chemically dispersed oil when effects are based on the amount of oil in water ($TPH_{(resolved)}$). This is clearly demonstrated when one examines

the constant exposure LC50 values. For the test species, these TPH-based LC50 values are very similar, not only between individual oils with and without the use of dispersant, but also among the four different oils. Lethal loading effect concentrations occur over a wide range, spanning approximately three orders of magnitude for all species, all oils. If focusing on actual exposures using $TPH_{(resolved)}$, the range of LC50s is reduced significantly. The smaller range is driven by the relative sensitivities of the organism, whereas the range in LL50s reflects both the organism sensitivity, but moreso the range of rates at which the oil enters the water.

Comparing toxicity of constant and spiked exposures. For constant exposure tests, the responses are in the range of 0.1 to 2 ppm $TPH_{(resolved)}$ among all test species (Table 1). For spiked exposure tests, the majority of the responses range from 2 to 20 ppm $TPH_{(resolved)}$, reflecting the extent to which spiked exposures lessen the toxicity of oil compared to constant exposures. Without dispersant, and without using a higher mixing rate, the authors could not get enough oil into the water to have exposures significant enough to cause mortality. Thus, the authors can report LC50s only as "greater than" the maximum test concentration, which usually was a no-effect exposure. Since this no-effect exposure value is less than exposure concentrations that could only be achieved by use of dispersants, they are not able to directly compare the toxicity of chemically and physically dispersed oil under spiked exposures. However, the LC50 values are expected to be similar to those obtained in the chemically dispersed, spiked exposure tests, and explained by TPH exposure concentrations as were the other tests.

Figure 3 allows us comparison of the magnitude of the LC50s from continuous and spiked exposures, and evaluation of the relative sensitivities of the test species. For most comparisons, focus needs to be on the chemically dispersed oil tests, since dispersants were required to get sufficient oil into the water for a full exposure-response assessment under spiked exposure conditions. Overall, constant exposures were 4 to 1,000 times more toxic than the spiked exposures, with the maximum difference shown by the gulf mysid, *M. bahia*, LC50s of 0.11 ppm for constant exposure to chemically dispersed weathered Kuwait crude and 111 ppm for spiked exposures. Within the confidence intervals for all the tests reported in Table 1, there were only minor differences in species sensitivity under constant exposures. For spiked exposures, the kelp mysid and oyster larvae appear to be more sensitive than the gulf mysid and the fish species.

Dispersant tests. Results of toxicity tests conducted with dispersants only are reported in Table 1, and results are summarized in Figure 4. During 48- to 96-hour constant exposure dispersant tests, the oyster embryo was the most sensitive species, and the fish were the least sensitive (with the yolk-sac turbot larvae less sensitive than the juvenile silversides). In the spiked (declining, 107 minute half-life) or pulsed (2-hour exposure) tests, the oyster embryos and the yolk-sac turbot larvae are again the most and least sensitive species, respectively. However, both crustaceans were much less sensitive than the juvenile silversides. Similar responses are demonstrated with the species tested by Singer, *et al.* (1991, 1993). There is a clear difference in the sensitivity of organisms exposed to dispersant under environmentally realistic, spiked (declining) conditions compared to more standard 48- to 96-hour constant exposures. Mollusks are 4 to 8 times less sensitive, crustaceans are 19 to more than 35 times less sensitive, and yolk-sac fish larvae are more than 14 times less sensitive under spiked exposure conditions. Juvenile topsmelt (*Atherinops affinis*) are 3 times less sensitive; however, differences in sensitivity with silversides are less pronounced.

As with the dispersed oil tests, dispersants showed relatively minor differences between the sensitivity of the standard EPA test species (*C. gigas, M. bahia, M. beryllina*) and the organisms used by the state of California (*H. rufescens, H. costata, A. affinis*). Decisions regarding the use of dispersants in spill response normally provide for a considerable degree of conservatism, which incorporates these small species-to-species differences in sensitivity. Therefore, decisions based on toxicity data from an EPA-recommended species should be protective of local species.

Dispersant use during oil spill response in open water areas, where mixing and dilution are maximized and exposure durations are brief, has considerably less potential for adverse impact to aquatic organisms than the hazard characterized by standard, continuous exposure toxicity tests. There are limited numbers of accurate and reliable field data on concentrations and durations of

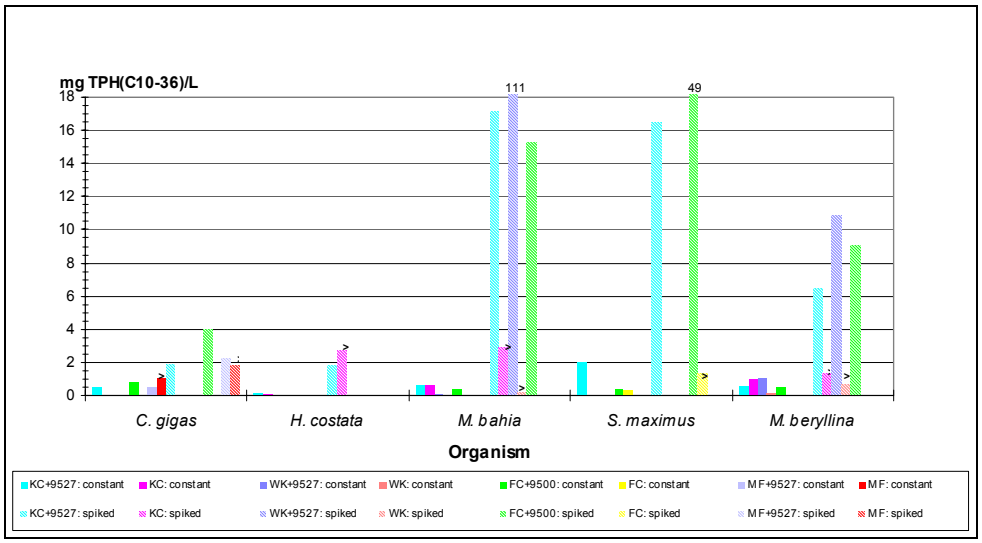

Figure 3. Plot of LC50s from toxicity tests using WAFs prepared with physically or chemically dispersed oils, five test species, and constant or spiked exposure regimes.

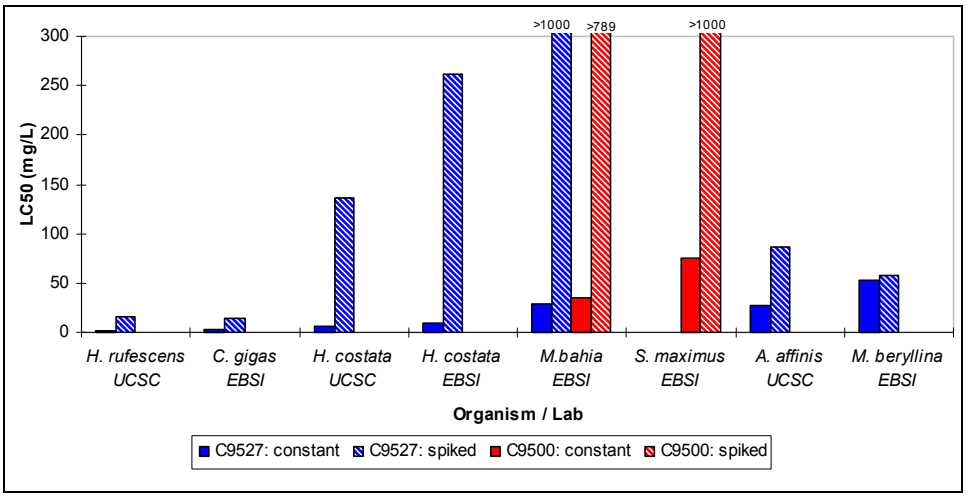

Figure 4. Plot of LC50s for continuous and spiked exposure toxicity tests with five test species used in tests at the authors' laboratory (noted with EBSI) and two additional species tested at the University of California Santa Cruz (noted with UCSC) as reported by Singer *et al.* (1993).

petroleum hydrocarbons in the water column near oil spills. Findings reported by Neff (1991) from extensive studies surrounding the *Exxon Valdez* oil spill and by Lunel (1994) from controlled, experimental spills in the North Sea show that very few, if any, of the measured water samples exceed the LC50 concentrations for the highly sensitive species and life stages tested in our study.

Conclusions

This series of tests have shown that the LC50s for physically and chemically dispersed oil are similar when calculated using the measured $TPH_{(resolved)}$ values for the WAFs. Dispersants were effective in getting more TPH into the water column compared to WAFs prepared with oil alone. However, dispersant did not alter the toxicity of the oils, as demonstrated by the similar LC50s for both chemically and physically dispersed crude oil under constant exposures, when considering data from each test species individually. This indicates that toxicity is explained by the TPH component in test solutions and not the added dispersant.

Standard toxicity test methods using 48- to 96-hour continuous exposures overestimate toxicity of oil, chemically dispersed oil, and dispersant compared to more environmentally realistic spiked exposures. Since the decision to approve dispersant use routinely incorporates available toxicity data as a means to assess potential ecological impacts, the relevancy of those data should be taken into account. Potential effects from short-term exposures common to open-water spills will be considerably less than long-term, continuous exposures. Organisms that differ phylogenetically may exhibit different responses, as observed with the juvenile fish species as compared to yolk-sac fish or invertebrates. Regardless, standard test species (*C. gigas, M. bahia, M. beryllina*) demonstrate responses similar to common, local test species (*H. rufescens, H. costata, A. affinis, S. maximus*) while encompassing the range of effects observed. The authors' data begin to define the range of sensitivity among common, sensitive test species and their sensitive life stages. Information from these types of species should be sufficient to characterize the potential for adverse effects when developing spill response options.

References

1. ASTM (American Society for Testing and Materials). 1994. Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs, E 724-89. *Annual Book of ASTM Standards 11.04.* American Society for Testing and Materials, Philadelphia, PA. pp. 430–471.
2. Bragin, G.E., and J.R. Clark. 1995. *Chemically Dispersed Crude Oil: Toxicity to Regional and National Test Species Under Constant and Spiked Exposures*. MSRC Technical Report Series 95-023. Marine Spill Response Corporation, Washington, DC.
3. Bragin, G.E., J.R. Clark, and C.B. Pace. 1994. *Comparison of Physically and Chemically Dispersed Crude Oil Toxicity to Both Regional and National Test Species Under Continuous and Spiked Exposure Scenarios.* MSRC Technical Report Series 94-015. Marine Spill Response Corporation, Washington, DC.
4. Coelho, G.M., G.E. Bragin, D.V. Aurand, J.R. Clark, and D.A. Wright. 1995. Field and Laboratory Investigations of the Toxicity of Physically and Chemically Dispersed Crude Oil. *Proceedings,* 18th Arctic and Marine Oil Spill (AMOP) Conference. Environment Canada, Ottawa, Ontario. pp. 1117–1130.
5. EPA (U.S. Environmental Protection Agency). 1987. Mysid Shrimp Acute Toxicity Test. USEPA CFR Toxic Substances Control Act Test Guidelines, part 797 Environmental Effects Testing Guidelines, Subpart B. Aquatic Guidelines, 797.1930. Federal Register 50(188) 1985 as revised by 52(97).
6. EPA (U.S. Environmental Protection Agency). 1994. Subpart J: Use of Dispersants and Other Chemicals, Appendix C: Section 3, Revised Standard Dispersant Toxicity Test. 40CFR Part 300 Federal Register (0915/94). 59(178):47453–47473.
7. Finney, D.J. 1971. *Probit Analysis*, 3rd ed. Cambridge University Press, London, England.
8. Hamilton, M.A., R.C. Russo, and R.V. Thruston. 1977. Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. *Environmental Science and Technology*. 11:714–719.

9. Lewis, A., and D. Aurand. 1997. *Putting Dispersants to Work: Overcoming Obstacles*. An issue paper prepared for the 1997 International Oil Spill Conference. American Petroleum Institute, Washington, DC.
10. Lunel, T. 1994. Dispersion of a Large Experimental Slick By Aerial Application of a Dispersant. *Proceedings,* 17th Arctic and Marine Oil Spill (AMOP) Conference. Environment Canada, Ottawa, Ontario. pp 951–977.
11. Neff, J.M. 1991. *Water Quality in Prince William Sound and the Gulf of Alaska*. Arthur D. Little, Cambridge, MA.
12. Pace, C.B., and J.R. Clark. 1993. *Evaluation of a Toxicity Test Method Used for Dispersant Screens in California.* MSRC Technical Report Series 93-028. Marine Spill Response Corporation, Washington, DC.
13. PARCOM (Paris Commission). 1994. *Fish Ring-Test*. A draft procedure for the determination of the acute toxicity of a test substance to marine fish larvae.
14. Pauwels, S.J., and J.R. Clark. 1993. Overview of International Oil Spill Dispersant Toxicity Testing Requirements. *Proceedings,* 1993 International Oil Spill conference. American Petroleum Institute, Washington, DC. pp. 803–804.
15. Singer, M.M., D. Aurand, G.E. Bragin, J.R. Clark, G.M. Coelho, M.S. Sowby, and R.S. Tjeerdema. In press. Standardization of Preparation and Quantification of Water-Accommodated Fraction of Petroleum for Toxicity Testing. *Marine Pollution Bulletin.*
16. Singer, M.M., D. Aurand, G.M. Coelho, G.E. Bragin, J. R. Clark, M.R. Sowby, and R. S. Tjeerdema. 2001a. Making, Measuring, and Using Water Accommodated Fractions of Petroleum for Toxicity Testing. *Proceedings,* 2001 International Oil Spill Conference. American Petroleum Institute, Washington, DC.
17. Singer, M.M., S. George, D. Benner, S. Jacobson, R.S. Tjeerdema, and M.L. Sowby. 1993. Comparative Toxicity of Two Oil Dispersants to the Early Life Stages of Two Marine Species. *Environmental Toxicology and Chemistry*. 12:1855–1863.
18. Singer, M.M., S. George, D. Benner, S. Jacobson, R.S. Tjeerdema, and M.L. Sowby. 2001b. Toxicity of Physically and Chemically Dispersed Oil to West Coast Test Species Using CROSERF Toxicity Test Methods. *Proceedings,* 2001 International Oil Spill Conference. American Petroleum Institute, Washington, DC.
19. Singer, M.M., D.L. Smalheer, and R.S. Tjeerdema. 1990. A Simple Continuous-Flow Toxicity Test System for Microscopic Life Stages of Aquatic Organisms. *Water Research*. 24:899–903.
20. Singer, M.M., D.L Smalheer, R.S. Tjeerdema, and M. Martin. 1991. Effects of Spiked Exposure to an Oil Dispersant on the Early Life Stages of Four Marine Species. *Environmental Toxicology and Chemistry*. 10:1367–1374.
21. Weber, C.I. 1993. *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 4th ed. EPA/600/4-90/027F. Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.