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ACUTE TOXICITY OF EIGHT LABORATORY-PREPARED GENERIC DRILLING FLUIDS TO MYSIDS (*Mysidopsis bahia*)

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ERRATA

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Page 6, line 6.	"...same as those...."
Page 10, line 10.	"...oil-free drilling fluids,...."
Page 12, line 5.	"CENTEC[a]"
Page 12, line 18.	"[a]Centec Analytical Service...."
Page 14, line 9.	"Polyanionic Cellulose"
Page 18, line 11.	"Definitive #2 5% 18% 18% - 42 80% 100% - - - -"

DISCLAIMER

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FOREWORD

The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the marine environment be formulated on a sound scientific basis. Accurate information describing concentration-response relationships for organisms and ecosystems under varying conditions is required. The Environmental Research Laboratory, Gulf Breeze, contributes to satisfying this information requirement through research programs aimed at determining:

- the effects of toxic organic pollutants on individual species and communities of organisms.
- the effects of toxic organics on ecosystems processes and components.
- the significance of chemical carcinogens in the estuarine and marine environments.

This report addresses the acute toxicity of eight generic drilling fluids to mysids, *Mysidopsis bahia*, and the toxicity of two of the fluids containing a mineral oil to these organisms. The tests were conducted in response to a request from the Office of Water Regulations and Standards and tested according to procedures prescribed by the Office.

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ABSTRACT

Acute toxicity tests were conducted during August-September 1983 with eight laboratory-prepared generic drilling fluids (also called muds) and mysids (Mysidopsis bahia) at the U.S. Environmental Protection Agency's Environmental Research Laboratory, Gulf Breeze, Florida. Two of the drilling fluids were tested at the Environmental Research Laboratory, Narragansett, Rhode Island, to confirm the validity of the tests conducted at Gulf Breeze.

The test material was the suspended particulate phase (SPP) of each drilling fluid. The SPP was prepared by mixing volumetrically 1 part drilling fluid with 9 parts seawater and allowing the resulting slurry to settle for one hour. The material that remained in suspension was the SPP.

Toxicity of the SPP of the drilling fluids ranged from a 96-hour LC50 (the concentration lethal to 50% of the test animals after 96 hours of exposure) of 2.7% for a KCl polymer mud to 65.4% for a lightly treated lignosulfonate mud. No median effect (50% mortality) was observed in three drilling fluids — a non-dispersed mud, a spud mud, and a seawater-freshwater gel mud.

Two of the generic drilling fluids to which mineral oil had been purposely added were also tested at Gulf Breeze. The addition of the mineral oil increased the acute toxicity of each fluid to mysids. When 1% mineral oil was added, the 96-hour LC50 changed from 51.6% to 13.5% for fluid #2, a seawater lignosulfonate mud, and from 29.3% to 7.1% for fluid #8, a freshwater lignosulfonate mud. Addition of 5% and 10% mineral oil further increased toxicity.

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ACKNOWLEDGMENTS

The cooperation of the Environmental Research Laboratory, Narragansett, Rhode Island, is gratefully acknowledged. Mr. S. Schimmel, U.S. Environmental Protection Agency, served as coordinator for the two tests conducted there; Messrs. T. Coffee and D. Wayne of the Edgerton Research Laboratory, New England Aquarium, Boston, Massachusetts, performed the tests under contract to the Narragansett laboratory.

At Gulf Breeze, personnel support was provided by Georgia State University, Cooperative Agreement CR809370, for Mr. R. Parrish during most of the testing program and by the University of West Florida, Cooperative Agreement CR807417, for Mr. R. Montgomery and Mrs. S. Macauley during the entire testing program.

INTRODUCTION

The purpose of this study was to determine the acute toxicity of eight laboratory-prepared generic drilling fluids to mysids (*Mysidopsis bahia*) according to methodology prescribed by the Effluent Guidelines Division, Office of Water Regulations and Standards, U.S. Environmental Protection Agency (EPA). Toxicity tests with all fluids were conducted at the EPA Environmental Research Laboratory, Gulf Breeze (ERL/GB), Florida, during August–September 1983, and two confirmatory tests were conducted at the EPA Environmental Research Laboratory, Narragansett, Rhode Island, during the same time period.

Acute toxicity tests were also conducted at ERL/GB during September-October 1983 with two of the generic drilling fluids that had been purposely contaminated with mineral oil. The purpose was to determine the toxicity of mineral oil in representative drilling fluids.

The generic drilling fluid concept was developed jointly by EPA's Region II and industry to provide EPA information on the impact of drilling fluids and components of the fluids released into the environment without requiring each drilling operator to perform repetitious toxicity tests and chemical analyses. The eight generic drilling fluids include virtually all water-based fluids used on the U.S. Outer Continental Shelf. Only major components of the generic fluids are specified and additional information concerning the effects of speciality additives must be submitted to EPA prior to their discharge (Petrazzulo, 1983). The generic fluid concept is now being employed by EPA Regional Offices involved in the permitting process (EPA, 1983).

MATERIALS AND METHODS

A. Drilling Fluids

The eight laboratory-prepared generic drilling fluids to be tested were received at ERL/GB during June and July 1983. They were sent via commercial airlines by each formulator. Each drilling fluid was contained in four 3.5-gallon plastic buckets with lids tightly sealed. The buckets were shipped in plastic ice chests, some of which contained wet ice or "blue ice." All buckets were cool to the touch when they arrived at ERL/GB. Upon receipt, each bucket of fluid was immediately placed in a large walk-in cooler with temperature maintained at approximately 4°C until the contents of each bucket were combined, mixed, and recontainerized. The purpose of mixing was to assure homogeneous samples for testing and/or chemical analyses. Mixing was accomplished by emptying each bucket of a fluid into a large plastic container. The fluid was stirred by hand with a large Teflon® paddle as approximately half of the contents of each bucket were alternately emptied. The fluid was then stirred for an additional 2-5 minutes, and was poured back into three of the original buckets that had been washed with tap water (except for the first fluid received, EPA-83-00800, which was put into new buckets). Drilling fluid equivalent to the contents of approximately one bucket (about 11 liters) was placed in new 1-liter plastic jars. All containers were labeled and capped (new lids were used on buckets because the original lids were destroyed when opened) after the air space above the fluid was flooded with nitrogen. The caps/lids were securely fastened, and the containers were placed in the walk-in cooler.

A detailed account of the treatment of each drilling fluid from the date of receipt to testing and/or transshipment is given in Table 1; the source and composition of each fluid are listed in Table 2. Appendix A contains a description of the physical and chemical characteristics of the mineral oil added to two of the generic drilling fluids.

B. Reference Toxicant

Sodium lauryl sulfate (dodecyl sodium sulfate) was used as a reference toxicant for the positive control. The chemical used was manufactured by Sigma Chemical Company, No. L-5750, Lot. 42F-0039, and was approximately 95% pure. Chemical from the same lot was used in the positive control tests at Gulf Breeze and Narragansett.

C. Methods for Drilling Fluid Tests

Test methods followed those proposed by Petrazzuolo (1983) with the following exceptions:

(1) Natural seawater from the ERL/GB seawater system was used instead of artificial seawater. The natural seawater was pumped from Santa Rosa Sound and filtered through sand and a 5-micrometer fiber filter; salinity was controlled at 20±2 parts per thousand by the addition of deionized water, and temperature was controlled by a commercial chiller;

(2) 5±1-day-old mysids were used instead of 4±1day-old mysids;

(3) Test mixtures were aerated; and

(4) For the mineral oil tests, glassware was washed with petroleum ether to assure removal of the oil.

At the outset, one or more 1-liter jars of the drilling fluid(s) to be tested were selected impartially. The fluid was mixed in the jar for at least 30 minutes by using a 1,600-rpm electric stirrer which turned a four-blade stainless steel stirrer. While the fluid was stirring, seawater was aerated in a container in an ice-bath.

To prepare the suspended particulate phase of the drilling fluid, 800 milliliters of the chilled seawater was added to a 2-liter Erlenmeyer flask. Then, 200 milliliters of the well-stirred drilling fluid was added to the flask. More seawater (1,000 mililiters) was added to bring the contents of the flask to the 2-liter mark. This 1-part fluid:9-part seawater slurry was placed on a magnetic stirrer and mixed for at least 5 minutes. The pH was measured and, if necessary, glacial acetic acid was added to bring the pH of the slurry within 0.2 pH units of the seawater, as directed by the test method. (If too much acid was added, sodium hydroxide was added as needed to increase pH.) The slurry was mixed for an additional 5 minutes if acid was added and then allowed to settle for 1 hour. The suspended phase (that is, all the liquid and suspended particulate above the settled or solid phase) was then decanted into a separate container, and pH and dissolved oxygen (DO) concentration were measured. If necessary, pH was again adjusted. Also, if DO was less than 65% of saturation, the suspended particulate phase was aerated, usually for 5 minutes. The appropriate volume of this 100% suspended particulate phase or seawater or both was added to a 2-liter Carolina culture dish (the total volume in each dish was 1 liter) to prepare the test and control mixtures. The mixtures were then stirred for approximately 30 minutes; the DO, pH, temperature and salinity were measured; and test animals were added to the dishes.

The number of animals exposed to a drilling fluid and the number of replicates varied. For range-finding tests, 10 mysids were added to each of 4 concentrations (100%, 50%, 10%, and 1% suspended particulate phase) and a seawater control, none of which was replicated. For definitive tests, 20 mysids were added to a seawater control and each of 5 concentrations (except only 3 concentrations were tested in those cases where no median effect, that is, 50% mortality, occurred in 100% suspended particulate phase in the range-finding test). Three replications of each treatment gave a total of 60 animals per treatment. For all tests, the animals in each dish were placed in holding cups fabricated by gluing a collar of 363-micrometer mesh nylon screen to a 15-centimeter wide glass Petri dish with silicone sealant. The nylon screen collar was approximately 5 centimeters high.

After measurement of water quality characteristics (DO, pH, salinity, and temperature) and addition of animals, the dishes were stacked three-high, with a cover on the top dish, and placed in an incubator. The temperature controller was set at 21°C and the light controller at 14 hours light:10 hours dark. All treatments were aerated at a volume estimated to be 50-140 cubic centimeters/minute during the tests. Air was delivered to each dish through polyethylene tubing (0.045-inch inner diameter and 0.062-inch outer diameter) by a small aquarium pump.

Water quality was measured at 24-hour intervals, but the turbidity of most fluids prevented 24-hour observations of test animals. After 96 hours, the test was terminated. If the solution was turbid, the cups were flushed with seawater until the animals became visible. Live animals were then removed by pipette and counted. Quality assurance was ensured by counting the control animals, placing them back in the holding cup and flushing them with seawater, and then recounting them.

Test methods used at Narragansett also followed Petrazzuolo's (1983) proposed methods. A report on the Narragansett tests is maintained at the Environmental Research Laboratories, Gulf Breeze and Narragansett.

D. Methods for Reference Toxicant (Positive Control) Tests

Test methods were those used for the drilling fluid tests, except that the test material was prepared by weighing one gram of sodium lauryl sulfate on an analytical balance, adding the chemical to a 100-milliliter volumetric flask, and bringing the flask to volume with deionized water. The test mixtures were prepared by adding 0.1 milliliter of the stock solution for each part per million desired to one liter of seawater. The mixtures were stirred briefly, water quality was measured, animals were added to holding cups, and the test was begun. Incubation and monitoring procedures were the same as these for the drilling fluids.

E. Test Animals

Mysids (Mysidopsis bahia) for the drilling fluid and reference toxicant tests were cultured at the Gulf Breeze and Narragansett laboratories. In addition, some mysids used for testing at Gulf Breeze were purchased from a commerical supplier, the origin of whose stock was the same as the laboratory stock. All mysids (5±1 days old) were fed Artemia salina nauplii (32–48 hours post-hydration) during holding and testing.

F. Statistical Analyses

Mortality data from the drilling fluid tests and the reference toxicant (positive control) tests were subjected to statistical analyses. A 96-hour LC50 (the concentration lethal to 50% of the test animals after 96 hours of exposure) was calculated for each drilling fluid (if the mortality data were amenable) and for each reference toxicant test by using the moving average method (Kendall and Stuart, 1973, and Stephan, 1977). The 95% confidence limits were also calculated. For tests where control mortality was ≤10%, no correction was made and the uncorrected data were used to compute LC50's and 95% confidence limits. For one mineral oil-contaminated drilling fluid and three reference toxicant tests at Gulf Breeze, the control mortality was >10% and data were corrected by using Abbott's formula (Abbott, 1925). The data used in drilling fluid LC50 calculations are contained in Appendix B.

Data from the Gulf Breeze tests with the eight generic drilling fluids (Appendix B) were also analyzed by SAS®, based on the probit method (Finney, 1971). A correction was made for all tests in which there was control mortality.[1]

To estimate the association between the mineral oil content and toxicity, data were analyzed by using Spearman's coefficient of rank correlation (Steel and Torrie, 1980).

[1] Analyses performed by R. Clifton Bailey, U.S. EPA, Program Integration and Evaluation Staff (WH-586), Office of Water Regulations and Standards, Washington, DC 20460.

RESULTS AND DISCUSSION

A. Generic drilling fluids

The range of toxicity of eight laboratory-prepared generic drilling fluids to mysids was considerable. The 96-hour LC50's were from 2.7% suspended particulate phase (fluid #1) to 65.4% (fluid #7). An LC50 could not be calculated for three fluids — #4, #5, and #6 — because no median effect (50% mortality) occurred (Table 3). It should be noted that these tests were not designed to identify the constituents in drilling fluid #1 that caused it to be more toxic than the other fluids.

The acute toxicity of the drilling fluids was generally related to the weight of the suspended material in the suspended particulate phase of each fluid, except for fluid #1. For example, fluid #7 contained 17.12 grams of suspended particulate matter per liter of the suspended particulate phase and the 96-hour LC50 was 65.4%. The more toxic fluid #3 contained 25.12 grams of suspended matter per liter, with a 96-hour LC50 of 16.3% (Tables 4 and 5).

The response of the mysids to the reference toxicant, sodium lauryl sulfate, showed that the test animal populations were in suitable condition for the toxicity tests. In five tests, the 96-hour LC50's were from 3.4 ppm to 7.5 ppm (Table 3). These are in accord with the literature and with unpublished data from Gulf Breeze and a commercial testing laboratory. The reference toxicant LC50's obtained at Gulf Breeze and Narragansett were similar even though the brood stocks and natural seawater were different.

Water quality was generally acceptable. The most serious problem was dissolved oxygen (DO) concentration. The oxygen demand of some drilling fluids (#3 and #8, for example) was high. The suspended particulate phase had to be aerated before testing began, and even with aeration during the test, DO concentrations were low. In only a few instances, however, was DO less than the minimally acceptable 65% of saturation. (NOTE: Raw data sheets are maintained at ERL/GB).

Reproducibility of results from test to test at Gulf Breeze was excellent. With drilling fluid #1, for example, three different tests were conducted: a range-finding test, a definitive test (which was repeated because of an unacceptable reference toxicant test), and a second definitive test. The results showed similar concentration-response relationships in all three tests (Table 6).

The interlaboratory agreement of test data from Gulf Breeze and Narragansett was equally good. The validity of the Gulf Breeze tests were confirmed by tests with two drilling fluids (#1 and #5) at Narragansett. The 96-hour LC50 for fluid #1 was almost exactly the same at both laboratories (Table 7). The results of the tests with fluid #5 were similar: Narragansett observed no mortality in 100% suspended particulate phase, whereas Gulf Breeze recorded 12% mortality in that concentration. Considering the nature of the test material and other possible sources of variability, this represents a more than satisfactory duplication of test results.

The slight differences in computed median effect concentrations were not considered significant. Whether there was correction for control mortality or not, and whether the moving average method or the probit method was used, there was excellent agreement among the toxicity test results.

B. Mineral oil-contaminated drilling fluids

The addition of mineral oil to laboratory-prepared generic drilling fluids #2 and #8 dramatically increased their acute toxicity to mysids. When 1% mineral oil was added, the 96-hour LC50 changed from 51.6% to 13.4%

for fluid #2 and from 29.3% to 7.1% for fluid #8. Addition of 5% and 10% mineral oil further increased toxicity (Table 8).

There was a significant negative correlation between mineral oil content and the 96-hour LC50 for each fluid; Spearmans's r = -0.976 with a probability ≤ 0.0001.

The response of the mysids to the reference toxicant was within an acceptable range, demonstrating that the test animals were in suitable condition (Table 9).

While the reproducibility of results from test to test was not as good as with oil-free drilling fluid, the test results did show similar concentration-response relationships (Table 10). The variation was probably caused by the volatility of the mineral oil and the need to aerate the suspended particulate phase before testing to achieve acceptable dissolved oxygen concentrations.

The presence of mineral oil in the generic drilling fluids did not adversely affect water quality. The DO, pH, salinity, and temperature were all within acceptable ranges during the tests.

LITERATURE CITED

Abbott, W.S. 1925. A Method of Computing the Effectivenesss of an Insecticide. J. Econ. Entomol. 18: 265-267.

Finney, D.J. 1978. Statistical Methods in Biological Assay, 3rd ed. Griffin Press, London. 508 pp.

Kendall, M.G. and Stuart, A. 1973. The Advanced Theory of Statistics, Vol. 3, 3rd ed., Hafner Publishing Co., New York, NY, pp. 342-430.

Petrazzuolo, G. 1983. Proposed Methodology: Drilling Fluids Toxicity Test for the Offshore Subcategory; Oil and Gas Extraction Industry. Technical Resources, Inc., Bethesda, MD 20817. DRAFT dated May 19, 1983.

Steel, R.G. and J.H. Torrie. 1980. Principles and Procedures of Statistics, 2nd ed. McGraw-Hill, New York, NY. 633 pp.

Stephan, C.E. 1977. Methods for Calculating an LC50. In: Aquatic Toxicity and Hazard Evaluation. ASTM STP 634, F.L. Mayer and J.L. Hamelink, Eds., American Society for Testing and Materials, Philadelphia, PA, pp. 65-84.

U.S. Environmental Protection Agency. 1983. Issuance of Final General NPDES Permits for Oil and Gas Operations on the Outer Continental Shelf (OCS) of Alaska; Norton Sound and Beaufort Sea. Federal Register Vol. 48, No. 236, December 7, 1983, pp. 54881-54897.

TABLE 1. Treatment of eight generic drilling fluids received at U.S. EPA, Gulf Breeze, Florida.

			TRANSSHIPPED			
			CENTIC[a]		NARRAGANSETT	
GENERIC MUDS	RECEIVED	MIXED	SHIPPED	RECEIVED	SHIPPED	RECEIVED
EPA-83-001	13 July 83	14 July 83	14 July 83	15 July 83	1 August 83	2 August 83
EPA-83-002	6 July 83	8 July 83	12 July 83	13 July 83	——	——
EPA-83-003	16 July 83	18 July 83	21 July 83	22 July 83	——	——
EPA-83-004	19 July 83	20 July 83	21 July 83	22 July 83	——	——
EPA-83-005	8 July 83	11 July 83	14 July 83	15 July 83	18 July 83	19 July 83
EPA-83-006	8 July 83	14 July 83	14 July 83	15 July 83	——	——
EPA-83-007	6 July 83	8 July 83	12 July 83	13 July 83	——	——
EPA-83-008	28 June 83	29 June 83	7 July 83	8 July 83	18 July 83	19 July 83[b]

[a] Centic Analytical Service, Salem, Virginia. For chemical analyses.

[b] Not tested at Narragansett.

TABLE 2. Source and reported composition of eight generic drilling fluids received at U.S. EPA, Gulf Breeze, Florida.

		Composition	
Drilling Fluid	Source	Component	Concentration
EPA-83-001, KCl Polymer Mud	Chromalloy	KCl	50.0 grams (g)
		Drispac (Super-Lo)	0.5 g
		X-C Polymer	1.0 g
		Barite	283.2 g
		Starch	2.0 g
		Seawater	257.6 milliliters (ml)
EPA-83-002,	IMCO Services	Attapulgite	30.0 pounds per barrel (ppbbl)
Seawater Lignosulfonate Mud		Chrome Lignosulfonate	15.0 ppbbl
		Lignite	10.0 ppbbl
		Polyanionic Cellulose	0.25 ppbbl
		Caustic	To pH 10.5-11.0
		Barite	To bring mud weight to 17-18 pounds per gallon (ppg)
		Seawater	As needed
EPA-83-003, Lime Mud	Hughes	Benitonite	20.06 g
		Lime	5.01 g
		Barite	281.81 g
		Chrome Lignosulfonate	15.04 g
		Caustic	1.00 g
		Lignite	8.02 g
		Distilled water	257.04 ml
EPA-83-004, Non-dispersed mud	Newpark Drilling Fluids	Bentonite	13.0 ppbbl
		Acrylic Polymer (for suspension)	0.5 ppbbl
		Acrylic Polymer (for fluid loss control)	0.25 ppbbl
		Barite	190.7 ppbbl
		Deionized Water	299.6 ppbbl

(continued)

Table 2, continued.

EPA-83-005, Spud mud	NL Baroid	Bentonite	12.5	ppbbl
		Lime	0.5	ppbbl
		Barite	50.0	ppbbl
		Seawater/Freshwater	1.0	bbl
		Caustic	To pH 10.0	
EPA-83-006, Seawater/Freshwater Gel Mud	Milchem	Bentonite	20.0	ppbbl
		Polyaninic Cellulose	0.50	ppbbl
		Sodium Carboxymethyl Cellulose	0.25	ppbbl
		Barite	20.0	ppbbl
		Sodium Hydroxide	To pH 9.5	
		Seawater/Freshwater, 1:1	As needed	
EPA-83-007, Lightly Treated Lignosulfonate Mud	Magobar Dresser	Bentonite	20.0	ppbbl
		Chrome Lignosulfonate	5.0	ppbbl
		Lignite	3.0	ppbbl
		Soda Ash	1.0	ppbbl
		Carboxymethyl Cellulose	0.5	ppbbl
		Barite	178.5	ppbbl
EPA-83-008, Freshwater Lignosulfonate Mud	Dowell	Bentonite	15.0	g
		Chrome Lignosulfonate	15.0	g
		Lignite	10.0	g
		Carboxymethyl Cellulose	0.25	g
		Sodium Bicarbonate	1.0	g
		Barite	487.0	g
		Deionized Water	187.0	ml

TABLE 3. Results of acute toxicity tests with eight generic drilling fluids and mysids (Mysidopsis bahia). The tests were conducted at U.S. EPA, Gulf Breeze, Florida, during August-September 1983.

Drilling Fluid	Range-finding Test (median effect)	Definitive Test[a] (96-h LC50 & 95% CL)	Positive Control[a] (96-h LC50 & 95% CL)	Definitive Test[b] (96-h LC50 & 95% CL)
#1	>1% <10% SPP[c]	2.7% SPP (2.5–2.9)	5.8 ppm[d] (4.3–7.6)	3.3% SPP (3.0–3.5)
#2	>50% <100% SPP	51.6% SPP (47.2–56.5)	7.5 ppm (6.9–8.1)	62.1% SPP (58.3–65.4)
#3	>10% <50% SPP	16.3% SPP (12.4–20.2)	7.3 ppm (6.6–8.1)	20.3% SPP (15.8–24.3)
#4	No median effect in 100% SPP	12% mortality in 100% SPP	3.4 ppm (2.8–4.1)	—
#5	100% SPP	12% mortality in 100% SPP	Same as for #1	—
#6	No median effect in 100% SPP	20% mortality in 100% SPP	6.0 ppm (5.4–6.6)	—
#7	>50% <100% SPP	65.4% SPP (54.4–80.1)	Same as for #6	68.2% SPP (55.0–87.4)
#8	>10% <50% SPP	29.3% SPP (27.2–31.5)	Same as for #3	30.0% SPP (27.7–32.3)

[a] Calculations by moving average; no correction for control mortality unless stated.

[b] Calculations by SAS® probit; correction for all control mortality. Analyses performed R. Clifton Bailey, U.S. EPA, Program Integration and Evaluation Staff (WH-586), Office of Water Regulations and Standards, Washington, DC 20460.

[c] The suspended particulate phase (SPP) was prepared by mixing 1 part drilling fluid with 9 parts seawater. Therefore, these values should be multiplied by 0.1 in order to relate the 1:9 dilution tested to the SPP the whole drilling fluid.

[d] Corrected for 13% control mortality.

TABLE 4. Generic drilling fluid concentrations, based on volumetric preparations of the suspended particulate phase (SPP), and weight of suspended material in the SPP in tests at U.S. EPA, Gulf Breeze, Florida.

Fluid	Date SPP prepared	SPP (g/ℓ)	SPP 96-h LC50 μℓ/ℓ; ppm[a]	SPP 96-h LC50 μℓ/ℓ; ppm[b]
#1	15 Aug 83	10.54	27,000	2,700
#2	25 Aug 83	18.66	516,000	51,600
#3	22 Aug 83	25.12	163,000	16,300
#4	18 Aug 83	0.0018	—[c]	—
#5	15 Aug 83	0.1570	—[c]	—
#6	29 Aug 83	0.866	—[c]	—
#7	29 Aug 83	17.12	654,000	65,400
#8	22 Aug 83	32.19	293,000	29,300

[a] Based on 1:9 dilution.

[b] Corrected for 1:9 dilution.

[c] No median effect (50% mortality) occurred in 100% SPP.

Table 5. Relationship of the weight of suspended material in the suspended particulate phase of eight generic drilling fluids to toxicity tests conducted at U.S. EPA, Gulf Breeze, Florida.

Fluid	SPP (g/ℓ)	96-hour SPP[a] LC50
#4	0.0018	—[b]
#5	0.1570	—[b]
#6	0.866	—[b]
#1	10.54	2.7%
#7	17.12	65.4%
#2	18.66	51.6%
#3	25.12	16.3%
#8	32.19	29.3%

[a] The suspended particulate phase (SPP) was prepared by mixing 1 part drilling fluid with 9 parts seawater. Therefore, these values should be multiplied by 0.1 in order to relate the 1:9 dilution tested to the SPP of the whole drilling fluid.

[b] No median effect (50% mortality) occurred in 100% SPP.

TABLE 6. Results of three acute toxicity tests conducted with mysids (*Mysidopsis bahia*) and generic drilling fluid #1 at U.S. EPA, Gulf Breeze, Florida. Mortality data are given as percentages.

Test	Control	Exposure Concentration (SPP[a])									
		1	2	2.5	3	4	5	7.5	10	50	100
Range-finding	10%	10%	-	-	-	-	-	-	100%	100%	100%
Definitive #1	8%	15%	-	18%	-	-	100%	100%	100%	-	-
Definitive #2	5%	11%	11%	-	42%	80%	100%	-	-	-	-

[a] The suspended particulate phase (SPP was prepared by mixing 1 part drilling fluid with 9 parts seawater). Therefore, these values should be multiplied by 0.1 in order to relate the 1:9 dilution tested to the SPP of the whole drilling fluid.

TABLE 7. Results of acute toxicity tests with mysids (*Mysidopsis bahia*) and two generic drilling fluids conducted at U.S EPA, Gulf Breeze, Florida, and Narragansett, Rhode Island, during August-September 1983.

Test Location	Drilling Fluid	96-hour SPP[a] LC50	95% Confidence Limits
Gulf Breeze	#1	2.7%	2.5-2.9%
	#5	No Median Effect[b]	—
Narragansett	#1	2.8%	2.5-3.0%
	#5	No Median Effect[b]	—

[a] The suspended particulate phase (SPP) was prepared by mixing 1 part drilling fluid with 9 parts seawater. Therefore, these values should be multiplied by 0.1 in order to relate the 1:9 dilution tested to the SPP of the whole drilling fluid.

[b] No median effect (50% mortality) occurred in 100% SPP.

TABLE 8. Comparative acute toxicity of two generic drilling fluids without and with mineral oil tested with mysids (*Mysidopsis bahia*) at U.S. EPA, Gulf Breeze, Florida, during August-October, 1983.

Drilling Fluid[a]	96-hour SPP[b] LC50	95% Confidence Limits
#2	51.6%	47.2-56.5%
#2-01	13.5%	11.1-16.9%
#2-05	1.8%	1.4-2.2%
#2-10	0.49%	0.39-0.62%
#8	29.3%	27.2-31.5%
#8-01	7.1%	5.7-9.0%
#8-05	0.90	0.74-1.1%
#8-10	0.76%	0.63-0.87%

[a] The two digits following the generic drilling fluid number indicate the percentage of mineral oil in the fluid.

[b] The suspended particulate phase (SPP) was prepared by mixing 1 part drilling fluid with 9 parts seawater. Therefore, these values should be multiplied by 0.1 in order to relate the 1:9 dilution tested to the SPP of the whole drilling fluid.

TABLE 9. Results of acute toxicity tests with mysids (*Mysidopsis bahia*) and two generic drilling fluids containing mineral oil. The tests were conducted at U.S. EPA, Gulf Breeze, Florida, during September-October 1983. All LC50's and 95% confidence limits were calculated by the moving average method.

Drilling Fluid	Range-finding Test (median effect)	Definitive Test (96-h LC50 & 95% CL)	Positive Control (96-h LC50 & 95% CL)
2-01	>10 <50% SPP[a]	13.5% SPP (11.1-16.9)	5.3 ppm (4.6-6.1)
2-05	>1 <10% SPP	1.8% SPP[b] (1.4-2.2)	7.1 ppm[c] (6.4-7.9)
2-10	<1% SPP	0.49% SPP (0.39-0.62)	7.1 ppm[c] (6.4-7.9)
8-01	>1 <10% SPP	7.1% SPP (5.7-9.0)	4.3 ppm (3.7-4.9)
8-05	>1 <10% SPP	0.90% SPP (0.74-1.1)	5.6 ppm[c] (5.0-6.4)
8-10	<1% SPP	0.76% SPP[c] (0.63-0.87)	5.3 ppm (4.6-6.1)

[a] The suspended particulate phase (SPP) was prepared by mixing 1 part drilling fluid with 9 parts seawater. Therefore, these values should be multiplied by 0.1 in order to relate the 1:9 dilution tested to the SPP of the whole drilling fluid.

[b] Corrected for 13% control mortality.

[c] Corrected for 15% control mortality.

TABLE 10. Results of three acute toxicity tests conducted with mysids (Mysidopsis bahia) and generic drilling fluid #8-05 (5% mineral oil) at U.S. EPA, Gulf Breeze, Florida. Mortality data are given as percentages.

		Exposure Concentration (%SPP[a])											
Test	Control	0.5	0.625	1.0	1.25	2.0	2.5	4.0	5.0	8.0	10	50	100
Range-finding	0	-	-	20%	-	-	-	-	-	-	100%	100%	100%
Definitive #1	17%	-	40%	-	63%	-	72%	-	95%	-	100%	-	-
Definitive #2	10%	30%	-	48%	-	88%	-	95%	-	100%	-	-	-

[a] The suspended particulate phase (SPP) was prepared by mixing 1 part drilling fluid with 9 parts seawater. Therefore, these values should be multiplied by 0.1 in order to relate the 1:9 dilution tested to the SPP of the whole drilling fluid.

APPENDIX A

CHEMICAL AND PHYSICAL CHARACTERISTICS OF THE MINERAL OIL USED IN THE LABORATORY-PREPARED GENERIC DRILLING FLUID TESTS

1. Mineral oil analysis reported by IMCO Services, drilling fluid #2

Boiling range (IBP-FBP)	500-610°F
Vapor pressure	0.008
Vapor density	>8
Solubility in water @100°F	30 ppm
Specific gravity	0.845
Percent volatile by volume (%)	100
Evaporation rate	<0.01
Flash point (Pensky-Martens)	255°F

2. Mineral oil analysis reported by Dowell, drilling fluid #8

Chemical name	Paraffin - base oil
Chemical family	Petroleum hydrocarbon
Formula	Complex misture of petroleum hydrocarbons
Boiling point, IBP, °F	500
FBP, °F	610
Vapor pressure (mm Hg), 20°C	0.008
Vapor density (air @ 1)	>8
Solubility in water @ 100°F, ppm	30
Specific gravity (water = 1)	0.845
Percent volatile by volume, %	100; evaporates slowly at 100°F
Evaporation rate (n-Butyl acetate = 1)	<0.01
Appearance and odor	Clear light color. Slight kerosenic odor
Gravity, °API at 60°F	35.8
Flash point, COC, °F	258 (Pensky - Martens 255°F)
Pour point, °F	5
Color, ASTM D 1500, Saybolt color	28
Viscosity, cSt at 40°C	4.2
SSU at 100°F	41.0

This oil is stable, but strong oxidants such as liquid chlorine, concentrated oxygen, and sodium or calcium hypochlorite should be avoided.

APPENDIX B

MORTALITY DATA

Test Location	Drilling Fluid	SPP[a] Test Concentration	Mysids Exposed	Mysids Killed	Percentage Mortality
Gulf Breeze	#1	Control	60	3	5
		1%	60	11	18
		2%	60	11	18
		3%	60	25	42
		4%	60	48	80
		5%	60	60	100
	#2	Control	60	4	7
		10%	60	3	5
		30%	60	9	15
		50%	60	9	15
		70%	60	49	82
		90%	40	39	98
	#3	Control	60	6	10
		10%	60	15	25
		20%	60	39	65
		30%	60	39	65
		40%	60	45	75
		50%	60	53	88

(continued)

[a] The suspended particulate phase (SPP) was prepared by mixing 1 part drilling fluid with 9 parts seawtaer. Therefore, these values should be multiplied by 0.1 in order to relate the 1:9 dilution tested to the SPP of the whole drilling fluid.

APPENDIX B, continued.

Test Location	Drilling Fluid	SPP[a] Test Concentration	Mysids Exposed	Mysids Killed	Percentage Mortality
Gulf Breeze	#7	Control	60	2	3
		20%	60	13	22
		40%	60	21	35
		60%	60	21	35
		80%	60	37	62
		100%	60	41	68
	#8	Control	60	1	2
		10%	60	0	0
		20%	60	11	18
		30%	60	31	52
		40%	60	42	70
		50%	60	56	93
Narragansett	#1	Control	60	1	2
		1.0%	60	1	2
		2.5%	60	5	8
		5.0%	60	5	100
		7.5%	60	60	100
		10%	60	60	100

(continued)

APPENDIX B, continued.

Test Location	Drilling Fluid	SPP[a] Test Concentration	Mysids Exposed	Mysids Killed	Percentage Mortality
Gulf Breeze	#2-01	Control	60	1	2
		2.5%	60	12	20
		5%	60	16	27
		10%	60	21	35
		20%	60	26	43
		40%	60	56	93
	#2-05	Control	60	8	13
		1%	60	22	37
		2%	60	37	62
		4%	60	50	83
		8%	60	57	95
		16%	60	60	100
	#2-10	Control	60	4	7
		0.25%	60	17	28
		0.5%	60	29	48
		1%	60	46	77
		2%	60	49	82
		4%	60	60	100

(continued)

APPENDIX B, continued.

Test Location	Drilling Fluid	SPP[a] Test Concentration	Mysids Exposed	Mysids Killed	Percentage Mortality
Gulf Breeze	#8-01	Control	60	5	8
		1.9%	60	6	10
		3.2%	60	15	25
		5.4%	60	28	47
		9%	60	31	52
		15%	60	44	73
	#8-05	Control	60	6	10
		0.5%	60	18	30
		1%	60	29	48
		2%	60	53	88
		4%	60	57	95
		8%	60	60	100
	#8-10	Control	60	9	15
		0.5%	60	23	38
		1%	60	43	72
		2%	60	57	95
		4%	60	60	100
		8%	60	60	100