EFFECTS OF SOLUBLE FRACTIONS OF USED LIGHT-WEIGHT LIGNOSULFONATE TYPE MUD AND HEXAVALENT CHROMIUM ON THE COMPLETE LARVAL DEVELOPMENT OF CRABS, RHITHROPANOPEUS HARRISII AND CALLINECTES SAPIDUS

by

Cazlyn G. Bookhout
*Robert Monroe
Richard Forward
John D. Costlow, Jr.
Duke University
Durham, NC 27706

and

*North Carolina State University
Raleigh, NC 27607

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Project Officer

Charles McKenney, Jr.
Environmental Research Laboratory
U.S. Environmental Protection Agency
Gulf Breeze, FL 32561

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
GULF BREEZE, FLORIDA 32561

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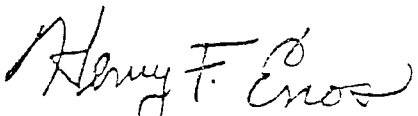
FOREWORD

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 environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The Environmental Research Laboratory, Gulf Breeze, contributes to this information 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 ecosystem processes and components,
- the significance of chemical carcinogens in the estuarine and marine environments.

This report describes the comparative toxicological effects of soluble fractions of drilling fluids and a common component of drilling fluids, chromium, on the complete larval development of two estuarine crab species. These data will be useful in assessing the possible effects of drilling fluids and their components on the marine and estuarine environment and biota. The study demonstrates a possible positive relationship between polycyclic aromatic hydrocarbons in the estuarine and coastal environment and cellular proliferative diseases in bivalve molluscs.



Henry F. Enos
Director
Environmental Research Laboratory
Gulf Breeze, Florida

ABSTRACT

The mud aqueous fractions (MAF) and suspended particulate phase (SPP) of lignosulfonate type mud were nontoxic to larvae during the complete larval development of Rhithropanopeus harrisii. Five percent MAF and SPP were not toxic to Callinectes sapidus. Survival of C. sapidus larvae decreased as concentrations of MAF and SPP increased from 5 to 50%. No larvae reached the 1st crab stage in 100% MAF and SPP. Statistical analyses of the data on survival, mortality and behavior are presented.

Survival of R. harrisii from hatching to 1st crab stage occurred in Na_2CrO_4 concentrations from 1.1 to 29.1 ppm. Estimated LC50 for complete zoeal development was 17.8 ppm Na_2CrO_4 and it was 13.7 ppm for development to 1st crab stage. A concentration of 1.1 ppm Na_2CrO_4 was nontoxic, while Na_2CrO_4 concentrations of 7.2 and 14.5 ppm were sublethal and concentrations of 29.1 to 58.1 ppm were acutely toxic. Low concentrations of Na_2CrO_4 caused an increase in swimming speed and high concentrations caused a decline.

Survival of Callinectes sapidus occurred in Na_2CrO_4 concentrations from 1.1 to 4.7 ppm. The LC50 for complete zoeal development was estimated to be 2.9 ppm Na_2CrO_4 and the LC50 for development to 1st crab stage was estimated to be 1.0 ppm Na_2CrO_4. Statistical analyses of the data on survival, duration and mortality of larvae are presented.

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SECTION 1

SUMMARY AND CONCLUSIONS

1. Survival of Rhithropanopeus harrisii from hatching to 1st crab stage occurred in mud aqueous fraction (MAF) concentration from 5% (5,000 ppm) to 100% (100,000 ppm) and in suspended particulate phase (SPP) concentrations from 5% (5,000 ppm) to 100% (100,000 ppm). The percent survival to megalopa and to 1st crab stage was 90% or over in seawater control and in 5, 25, 50 and 100% MAF and SPP in three replicate series of larvae tested.

2. Differential survival of Callinectes sapidus from hatching to 1st crab stage occurred in MAF and SPP concentrations from 5 to 50%. No larvae reached the 1st crab stage in either 100% MAF or 100% SPP. Five percent MAF and 5% SPP were nontoxic to larvae tested. Statistical analysis revealed for zoeal survival that there was approximately 4% decrease/10% increase in MAF @ 50% concentration (CONC), and for survival to 1st crab there was approximately 3% decrease/10% increase in MAF @ 50% CONC. For zoeal development and development from hatching to 1st crab, there was approximately 5% decrease in survival for a 10% increase in SPP near 50% SPP CONC.

3. There was no significant difference in duration in zoeal development and in hatch to 1st crab of C. sapidus in seawater control and in concentrations of MAF and SPP employed.

4. Mortality of larvae reared in 5 and 25% MAF was not significantly different from larval mortality in seawater control in any of the nine developmental stages of C. sapidus, but mortality of larvae reared in 50 and 100% MAF was significantly different from the control in every developmental stage. Even though larvae in zoeal stage I were most sensitive, larvae in zoeal stage II were also very sensitive.

5. Mortality of larvae in 5% SPP was not significantly different from mortality in the control in any of the nine developmental stages, but mortality of larvae reared in 50 and 100% SPP was significantly different from the control in every developmental stage of C. sapidus. In zoeal stage I 25% SPP was significantly different from the control at the 0.05 alpha level. As in the MAF experiment, although larvae in zoeal stage I were most sensitive, larvae in zoeal stage II were also very sensitive.

6. Blue crab larval behavior is affected by exposure to MAF and SPP with the general effect being a decline in swimming speed. A significant reduction was only observed in 100% MAF and 5, 25, 50 and 100% SPP.

7. Callinectes sapidus larvae could be in the vicinity of drilling operations during development and might be found in the upper turbidity plume, but the chances of many of the larvae remaining in the 3 m highly toxic zone, or even in the 15 m intermediate toxic zone, around the discharge source long enough to suffer mortality are very remote. If by chance a few 1st or 2nd stage zoeae of C. sapidus in the process of molting happened to be entrained within 15 m of discharge, they might be killed or receive irreversible stress, for these zoeae are extremely sensitive. Larvae in other stages could be affected, but not as quickly.

8. Survival of Rhithropanopeus harrisii from hatching to 1st crab stage occurred in Na_2CrO_4 concentrations from 1.1 to 29.1 ppm. No larvae reached the 1st crab stage in concentrations of 40.6, 46.4 and 58.1 ppm Na_2CrO_4. A concentration of 1.1 ppm Na_2CrO_4 was nontoxic to larvae tested. The estimated LC50 for complete zoeal development was 17.8 ppm Na_2CrO_4 and for development to 1st crab was 13.7 ppm Na_2CrO_4.

9. Statistical analysis of the data on R. harrisii duration revealed that there was 0.120 $\pm$ 0.021 days increase in duration of zoeal development from hatching to megalopa for each ppm added Na_2CrO_4, and that there was 0.122 $\pm$ 0.021 days increase in total duration time from hatching to 1st crab for each ppm added Na_2CrO_4.

10. There was differential mortality of R. harrisii larvae from concentrations of 1.1 to 58.1 ppm Na_2CrO_4. In 1.1 ppm Na_2CrO_4, there was no more mortality than in seawater control. Na_2CrO_4 concentrations of 7.2 ppm and 14.5 ppm were sublethal and those of 29 to 58 ppm were found to be acutely toxic.

11. Rhithropanopeus larval swimming speed was affected by exposure to Na_2CrO_4. The general pattern was for the swimming rate to be elevated with short-term exposure to concentrations from 1.1 to 29.1 ppm Na_2CrO_4 and with long-term exposure to low concentrations of 1.1 and 7.2 ppm Na_2CrO_4. Swimming rates were only depressed in later stages upon long term exposure to high Na_2CrO_4 concentrations of 14.5 and 29.1 ppm. Thus in general, low concentrations caused an increase in swimming speed and high concentrations caused a decline.

12. Survival of Callinectes sapidus from hatching to 1st crab stage occurred in Na_2CrO_4 concentrations from 1.1 to 4.7 ppm. There was better survival in 1.1 ppm than in seawater control, but there was differential survival from 1.1 to 7.2 ppm Na_2CrO_4. The LC50 for complete zoeal development was estimated to be 2.9 ppm Na_2CrO_4, and the LC50 for development from hatching to 1st crab was estimated to be 1.0 ppm Na_2CrO_4.

13. Statistical analysis of the data on C. sapidus duration revealed that there was 1.65 ± 0.29 days increase in duration of zoeal development from hatching to megalopa for each ppm added Na_2CrO_4, and that there was 1.31 ± 0.29 days increase in total duration time from hatching to 1st crab stage for each ppm added Na_2CrO_4.

14. There was significantly less mortality in 1.1 ppm Na_2CrO_4 than in seawater control. A Na_2CrO_4 concentration of 2.4 ppm was nontoxic. There was differential mortality from 4.7 to 7.2 ppm Na_2CrO_4. These concentrations are considered acutely toxic since less than 10% of C. sapidus larvae reached the 1st crab stage. Zoeae in zoeal stage III were extremely sensitive to 7.2 ppm Na_2CrO_4, and zoeae in zoeal stages III, IV and V were most sensitive to 4.7 ppm Na_2CrO_4. In seawater control, 1.1 and 2.4 ppm Na_2CrO_4, there was a highly significant increase in mortality in the megalopa stage over the previous stage.

15. For most discharges, the background level for chromium has been reported to be reached approximately 100 to 150 meters from the point of discharge. Within this area, entrained crab larvae would undoubtedly absorb Cr^{+6} more readily than Cr^{+3}, if both were present, and bioaccumulate chromium. It is very questionable, however, whether crab larvae would remain in the upper turbidity plume long enough to bioaccumulate enough chromium to kill the larvae or to produce sublethal stress. Hence, it is probable that chromium in drilling fluids is not likely to reduce the population of crab larvae and other planktonic organisms in the area around an oil well, except possibly in the immediate vicinity of the discharge pipe.

SECTION 2

RECOMMENDATIONS

1. Records of constituents of new drilling fluids should be kept on file with a central agency together with the log of the time and the amount of specific additives made at different depths. This information, should be made available to investigators who will conduct acute and chronic toxicity tests.

2. Approximately 90% of the main constituents of drilling fluids have been reported to be nontoxic, but some of the remaining 10% additives are toxic. Separate and joint toxicity studies should be made of the latter to determine if the components are nontoxic, less than additive, additive, or more than additive in toxicity, or antagonistic to one another following the experimental design of Sprague and Logan (1979).

3. There is a need for more detailed chemical analysis of trace metals in the suspended particulate fraction and the water-soluble fraction of drilling fluids after discharge into the ocean. Particular attention should be given to speciation of chromium if there is evidence that Cr^{+6} is present.

4. Residue analyses of planktonic organisms for trace metals should be made from the 150 meter zone around the discharge source and compared to comparable samples taken from outside. If there is evidence of bioaccumulation of trace metals by larvae or small crustaceans within the vicinity of the upper turbidity plume, would the bioaccumulated metals be passed through a food web to higher organisms?

5. Chronic tests on the effects of Cr^{+3} on the complete larval development of Rhithropanopeus harrisii and Callinectes sapidus should be made in order to make comparisons with the findings in the current investigation on the effect of Cr^{+6} on the development of the same species of crabs.

SECTION 3

GENERAL MATERIALS AND METHODS

Preliminary experiments were conducted to determine the range of concentrations of the Mud Aqueous Fraction (MAF) and the Suspended Particulate Phase (SPP) of (No. 4 Mud) lignosulfonate type mud with ferrochrome lignosulfonate added to use in definitive chronic toxicity studies on the development of Rhithropanopeus harrisii (Gould) and Callinectes sapidus Rathbun. Preliminary experiments were also performed to determine the concentrations of sodium chromate (Na_2CrO_4) to use in definitive chronic studies on the development of R. harrisii and C. sapidus.

The lignosulfonate type mud which was tested was sent to us in 5.7 liter polyethylene containers by the U.S. Environmental Protection Agency, Environmental Research Laboratory (ERL), Gulf Breeze, Florida. Upon arrival at the Duke University Marine Laboratory, Beaufort, N.C., the container was placed in a cold room where the temperature was 4°C. The mud was originally collected on July 22, 1980 just after it went through the shaker table and the cuttings were removed. The mud was collected at a depth of 3,735.9 meters (12,257 feet) with a density of 4.1 kg/3.785 liters (9.1 lb/gal), a viscosity of 58 sec/qt API and water content of 88.3%. Further analysis of the mud furnished by ERL-Gulf Breeze is given in TABLE 1.

TABLE 1. SUMMARY OF THE ANALYSES OF LIGHT-WEIGHT LIGNOSULFONATE TYPE MUD WITH FERROCHROME ADDED (NO. 4 MUD) FROM THE JAY, FLORIDA EXXON WELL BY DR. ROBERT SHOKES, SCIENCE APPLICATIONS (PERSONAL COMMUNICATION)

Metals %		Metals ppm		Total Resolved Aliphatics (µ g/l)	Total Resolved Aromatics (µg/l)	H_2O %
Al	8.00	Pb	40.2	17,000	27,300	88.3
Fe	3.19	Cr	96.4			
Ba	4.10	Zn	225.0			

The Mud Aqueous Fraction (MAF) was prepared following the methods of Neff et al., 1980 by mixing one part used drilling mud with nine parts of 20°/₀₀ seawater for experiments on R. harrisii larvae and 30°/₀₀ seawater for studies on C. sapidus larvae. The mixture was stirred thoroughly with an electric mixer and then allowed to settle for 20 hours. The dark colored aqueous layer was siphoned off for use in toxicity tests. For MAF toxicity tests on R. harrisii and C. sapidus larvae, 100% MAF was prepared by mixing 120 ml of undiluted mud with 1080 ml of seawater. Fifty percent MAF

was prepared by adding 150 ml of 100% MAF to 150 ml of seawater, 25% MAF was made by adding 75 ml of 100% MAF to 225 ml of seawater, and 5% MAF was prepared by adding 15 ml of 100% MAF to 285 ml of seawater.

Suspended Particulate Phase (SPP) was prepared following the methods of Neff et al. (1980). One hundred percent SPP was made by air mixing with compressed air one part undiluted mud with nine parts seawater (20°/oo salinity for R. harrisii and 30°/oo for C. sapidus) for one-half hour with manual stirring every 10 minutes. After aeration the suspension was allowed to settle for four hours before the supernatant was siphoned off for use in toxicity tests. The concentrations of SPP used for R. harrisii and C. sapidus were the same as those described for MAF. Our aeration was through two airstones connected by hoses to a compressed air line.

Source of Animals

Three ovigerous Rhithropanopeus harrisii furnished larvae for series designated as RhI, RhII and RhIII. The mother crab, which furnished larvae for RhI on September 30, 1980, was collected in the Neuse River near Havelock, N.C., and the crab which provided larvae for RhII on November 8, 1980 was found in the Newport River near Morehead City, N.C. It became ovigerous after it had been held in a habitat aquarium in the laboratory. Ovigerous R. harrisii which furnished larvae for RhIII on November 14, 1980 was collected in the vicinity of Fort Pierce, Florida, and was shipped air freight to Beaufort, N.C. There were sufficient larvae from one ovigerous crab to do one toxicity test on MAF and SPP fractions of No. 4 mud.

Adult R. harrisii which furnished larvae for series RhI to RhVI used in the sodium chromate experiments were collected near Morehead City and near Havelock, N.C. while they were in the refractory period during the fall of 1980 and winter of 1981. They were placed in an artificial habitat in the laboratory where the temperature of the water was 30°C and there was 14 h light and 10 h darkness per day. When the crabs became ovigerous, they were isolated in separate large finger bowls (19.4 cm diam) and maintained in a constant temperature cabinet at 25°C under a light regime of 12 h light and 12 h darkness until hatching occurred. The largest and most healthy hatches were selected for the experiments. Larvae for RhI and RhII hatched on January 20, 1981. The dates of hatching were February 17, 1981 for RhIII, February 23, 1981, for RhIV, March 18, 1981, for RhV and March 19, 1981 for RhVI.

In experiments on No. 4 mud, three ovigerous Callinectes sapidus were used to obtain larvae for series CsI, CsII and CsIII. Larvae of these series hatched on July 4, July 19 and July 24, 1981 from three ovigerous crabs which were collected in the Beaufort Inlet.

In experiments on Na_2CrO_4, three ovigerous Callinectes sapidus furnished larvae for series CsI, CsII and CsIII. Larvae of series CsI and II hatched on September 10 and CsIII hatched on September 11, 1981.

Long-Term Exposure of R. harrisii and C. sapidus Larvae to MAF and SPP

In each series of R. harrisii (RhI, RhII and RhIII), there were sufficient larvae for five bowls with 10 larvae in each bowl for seawater control and five bowls with 10 larvae each for 5%, 25%, 50%, and 100% MAF, and for 5%, 25%, 50%, and 100% SPP. Larvae were transferred daily to clean bowls containing fresh media. The diet was freshly hatched Artemia nauplii from Great Salt Lake, Utah. The salinity of the media was 20°/₀₀ and the bowls of larvae were maintained in a constant temperature cabinet of 25°C and in a light regime of 12 h light and 12 h darkness. When larvae molted to megalopa, each megalopa was placed in a separate compartment of a plastic box. Daily records were kept for the number of live and dead larvae as well as the stage of each dead larva. The time each larva molted to a megalopa and each megalopa molted to a first crab stage were also recorded.

When C. sapidus larvae were exposed to MAF and SPP, the same procedures as outlined above were used except the salinity of the media was 30°/₀₀ and larvae from the time of hatching until the first crab stage was reached were fed Arbacia embryos and Branchionus plicatilis, rotifers, plus Artemia nauplii after the second zoea had been reached.

Behavior of C. sapidus Larvae: Effects of Exposure to Drilling Mud MAF and SPP

Ovigerous C. sapidus were collected locally, and the larvae hatched in the laboratory. Larvae were reared in 30°/₀₀ filtered seawater at 25°C on a 12:12h:light dark cycle. The day after hatching, larvae were divided into groups of about 150 and each group exposed to a different set of conditions: 30°/₀₀ seawater alone, 5% MAF, 25% MAF, 50% MAF, 100% MAF, 5% SPP, 25% SPP, 50% SPP, and 100% SPP.. The MAF and SPP solutions were made up as previously described. The solutions were tested because they represent the entire range of concentration used for the developmental study. Each day the larvae were placed in new solutions and fed fertilized sea urchin eggs and Artemia nauplii. Larvae swimming speed was measured after 48 hours exposure in the middle of the light phase.

The behavior measured as indicative of stress was swimming speed. For these measurements larvae were placed in a cuvette positioned on the stage of a dissecting microscope, which was coupled to a closed circuit television system. The microscope illumination light was filtered to the near infra-red region. The larvae are insensitive to wavelengths in this region.

The general procedure was to place light-adapted larvae on the microscope stage, extinguish the room lights and after one minute record larval movements on video tape. In this way swimming was observed in darkness. The tapes were later analyzed for random swimming speeds. Although speeds measured during random swimming tend to underestimate the true rates (Forward, 1977), the obtained values still serve as an indicator of changes in activity (Forward and Costlow, 1976, 1978). Speeds for 20 larvae were measured for each hatch and condition. Since three separate hatches were tested, the total sample size for each condition was 60. Mean swimming speeds under the various conditions were compared by the Student's T test.

Long-Term Exposure of R. harrisii and C. sapidus Larvae to Sodium Chromate

Hexavalent chromium, Na_2CrO_4, was purchased from Fisher Scientific Company as Certified Anhydrous Sodium Chromate. A 58.09 ppt stock solution was prepared by dissolving a known weight of Na_2CrO_4 in glass distilled water and different concentrations were made from this stock solution by serial dilution. For experiments on the effect of Na_2CrO_4 on the development of R. harrisii, 1 ml of stock solution of 1.12 parts per thousand (ppt) (°/₀₀) (ml/l), 7.17 °/₀₀, 14.52 °/₀₀, 29.09 °/₀₀, 40.6 °/₀₀, and 58.09 °/₀₀ were added to 999 ml of 20°/₀₀ filtered seawater to give final concentrations from 1.12 parts per million (ppm) (mg/l) to 58.09 ppm. The concentrations of Na_2CrO_4 given in this manuscript are those determined by the Hazleton Laboratories America, Inc.

For experiments on the effect of Na_2CrO_4 on the development of C. sapidus, 1 ml of Na_2CrO_4 stock solution of 1.1 °/₀₀, 2.4 °/₀₀, 4.7 °/₀₀, and 7.2 °/₀₀ were added to 999 ml of 30°/₀₀ filtered seawater to give final concentrations from 1.1 ppm to 7.2 ppm Na_2CrO_4.

The methods for rearing larvae in a check series, 10 larvae per finger bowl (8.9 cm diam), was the same as previously described. Fresh solutions of Na_2CrO_4 were prepared every other day, placed in 1000 ml flask and dispensed by a 50 ml pipettor to finger bowls. Between daily changes of larvae to clean bowls, bowls of larvae were maintained in a constant temperature cabinet at 25°C and a light regime of 12 h light and 12 h darkness.

Behavior of R. harrisii larvae: Effects of Exposure to Na_2CrO_4

Ovigerous specimens of R. harrisii were collected from the Neuse River, North Carolina. The eggs hatched during the night after collection and the experiments begun the next morning. Larvae were reared in 20°/₀₀ filtered seawater, at 25°C on a 12:12 h light-dark cycle. Hatches were divided into groups of about 75 larvae and each group chronically exposed to a different set of conditions: 20°/₀₀ seawater alone, 1.2 ppm, 7.2 ppm, 14.5 ppm and 29.1 ppm sodium chromate. The sodium chromate solutions were made up as described previously. These concentrations were tested because they span the region from no effect upon larval mortality to concentrations that are almost totally lethal. Each day the larvae were placed in new solutions and fed Artemia nauplii. Larval behavior was monitored for all 4 zoeal stages on intermolt days. Furthermore, to avoid complications due to a possible biological rhythm in activity, larval behavior was measured between 4 to 10 h after the beginning of the light phase. The techniques for monitoring swimming speed are identical to those used for C. sapidus larvae.

Statistical analysis

The statistical methods used for analysis of larval development of R. harisii and C. sapidus subjected to treatment by MAF, SPP or Na_2CrO_4 are outlined in detail in two articles on Kepone (Bookhout et al., 1979; Bookhout et al., 1980). The mean swimming speeds under various conditions were compared by Student's T test.

Briefly, the methods relied on the angular transformation of survival and/or mortality percentages to stabilize the experimental error variance in order to use standard analysis of variance and regression techniques for the appropriate estimates and tests of significance. Durations of molting time expressed in days were also analyzed as "rates" (i.e. reciprocal days) since in some data sets the relationship between concentrations and rates was more nearly linear than the relationship with time in days.

In presenting the results of the several analyses mean values of survival and/or mortality were obtained from the retransformed mean values in the angular scale. Regression coefficients reflecting decreases in survival measured in the angular scale also were reexpressed as % decrease in surival/% change in concentration (or per 10 ppb increase when appropriate). Since the retransformation of the regression coefficients is non-linear (i.e. depends upon the level of survival) we usually chose the 50% survival as the point at which to reexpress as % decrease. However, in the data from the drilling muds that survival percentage was not achieved even the controls so the point of reexpression was chosen at 50% concentration (approximately 8-10% survival).

SECTION 4

EFFECTS OF SOLUBLE FRACTIONS OF USED LIGHT-WEIGHT LIGNOSULFONATE TYPE MUD ON THE COMPLETE LARVAL DEVELOPMENT OF CRABS, *Rhithropanopeus harrisii* AND *Callinectes sapidus*

INTRODUCTION

With the increase in number of oil wells and new leases for oil exploration along the Atlantic Coast, it is natural that the public would be concerned about the effect of the discharge of drilling fluids on marine fauna. Accordingly, this investigation will focus on the effect of a low-density lignosulfonate drilling mud with ferrochrome added (No. 4 mud) on the complete larval development of two crabs, a mud crab, *Rhithropanopeus harrisii* (Gould) and the blue crab, *Callinectes sapidus* Rathbun. The Jay Exxon well drilling fluid to be tested had a density of 9.1 lb/gal and came from a land based well in Florida. The samples of No. 4 mud were taken at a depth of 3735.9 m (12,257 feet) and were provided for this investigation by the United States Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida. Although the well was land based, it was believed that the chemical components and physical characteristics of the drilling fluid were similar to those in offshore wells.

Extensive tests on the effects of four to five used lignosulfonate drilling fluids on warm- and cold-water organisms from the Gulf of Mexico have been made by Neff *et al.* (1980, 1981), Carr *et al.* (1980), McCulloch *et al.* (1980) and Gerber *et al.* (1980). They used mud supplied by the American Petroleum Institute. For comparative purposes, therefore, there is pertinent information concerning the relative toxicity of spud mud with a density of 9.2 lb/gal, a low-density lignosulfonate (LWLS) drilling fluid with a density of 10.0 lb/gal, and a high density lignosulfonate (HWLS) with a density of 17.4 lb/gal.

Investigators have evaluated the toxicity of five components of whole mud following the classification originally proposed by Neff *et al.* (1980). The mud aqueous fraction (MAF) and suspended particulate phase (SPP) are two fractions which have been most intensively investigated in toxicity tests and are the two fractions tested in this investigation. The 100% MAF contains water-soluble and fine particulate fractions of 100,000 ppm mud in water. SPP resembles MAF but contains a higher concentration of particulates and a lower concentration of volatiles (Neff *et al.*, 1980). These two fractions are found in the upper plume of discharge and remain in the water column longer than other fractions (Ayers et al., 1980) and, hence, may be the fractions which might be expected to affect larvae of marine organisms.

Research on the environmental effects of drilling fluids is difficult because of the complexity of the fluids and the lack of homogeneity of samples from the same depth. Ninety percent of the major drilling fluid components are barite, primarily barium sulfate, clays such as sodium bentonite and calcium bentonite, lignosulfonates and lignite (Perricone, 1980). The fluids, however, may include additives, such as pH-control substances, bactericides, calcium removers, corrosion inhibitors, deformers, emulsifers, filtrate, reducers, flocculants, foaming agents, lubricants, shale-control inhibitors, thinners, dispersants, viscosifiers, and weighting agents (Richards, 1979). Furthermore, produced waters generally contain appreciable concentrations of inorganic salts and trace minerals compared to normal seawater.

Although most of the drilling mud is recovered and recycled, tons may be discharged as a turbity plume in the surface layers of water. Ray and Meek (1980) reported that 2,854 barrels of mud and cuttings were discharged, representing about 863,390 kg of solids, over an 85 day period on Tanner Bank, California. Hence, 10,000 kg or 10 metric tons would be discharged per day, 95% of which would be normal cuttings and 4% drilling mud.

At the present time, the impact of drilling fluids is incompletely known. Most investigations have been acute toxicity tests and they show that drilling fluids have little or no effect on adult marine organisms, but they reveal that larvae and juvenile invertebrates are sensitive to exposure to drilling fluids (Neff et al., 1980, 1981; Carr et al., 1980; Lees and Houghton, 1980; Gerber et al., 1980; Carls and Rice, 1981).

Although most of the components of drilling fluids are apparently nontoxic, some of the additives, such as the bactericide, pentachlorophenol (Tagatz et al., 1977), as well as paraformaldehyde, caprylalcohol and some surfactants are especially toxic (Sprague and Logan, 1979). Trace metals found within drilling fluids may also have detrimental effects (Liss et al., 1980; Hrudey and Eng, 1979).

Sprague and Logan (1979) investigated separate and joint toxicity to rainbow trout of substances used in drilling fluids for oil exploration in the MacKenzie delta. When seven most toxic components were added singly to a simulated fluid about half of the combinations showed joint action. In several preparations, however, antagonism apparently occurred. The research of Sprague and Logan (1979) illustrates the chemical complexity of one drilling mud used in the MacKenzie delta. The potential effects of chemicals, however, will differ with each type of mud in well drilling operations, with cutting composition related to type of substrate drilled, with well depth, temperature generated, etc. (Richards, 1979).

As far as known, there have been no investigations on the effect of the soluble fractions of whole mud on the complete larval development of crabs, such as the suspended particulate phase (SPP) and mud aqueous fraction (MAF), which would be found in the upper plume of discharges.

The objectives of the current investigation were, therefore, to determine the range of concentrations of MAF and SPP of used lignosulfonate type mud with

ferrochrome added (No. 4 mud) which would affect swimming behavior, survival and duration of development of the mud crab, Rhithropanopeus harrisii, and the blue crab, Callinectes sapidus, from the time of hatching until the 1st crab stage is reached. Further objectives were to ascertain the concentrations of MAF and SPP which were nontoxic, sublethal and acutely toxic, the sublethal effects, and the most sensitive periods of development of the two species.

RESULTS

Survival and Duration of Rhithropanopeus harrisii Larvae

TABLE 2 gives the percent survival from hatching to megalopa and to 1st crab stage and the mean duration in days of zoeal and megalopa development, as well as the time from hatching to 1st crab stage of series Rh-I, Rh-II and Rh-III reared in seawater control, four concentrations of MAF and four concentrations of SPP. TABLE 3 gives average survival and duration data of the three series reared from hatching to 1st crab stage. From the results tabulated, it can be noted that the percent survival to megalopa and to 1st crab stage is 90% or over in seawater control and in all concentrations of MAF and SPP. There is no consistent reduction in survival in concentrations of MAF or SPP compared to survival in seawater control. TABLE 3 also shows that the average duration in days from hatching to 1st crab stage is fairly uniform in seawater control and in all concentrations of MAF and SPP. Percent mortality in developmental stages of each of three series of R. harrisii reared in seawater control and different concentrations of MAF and SPP is listed in TABLE 4, whereas TABLE 5 gives the average percent mortality of the three series.

Survival of Callinectes sapidus Larvae

The percent survival from hatching to megalopa and to 1st crab for C. sapidus is given for replicate series reared in different concentrations of MAF and SPP in TABLE 6. The average percent survival of all series reared in seawater control and four concentrations of MAF and SPP is listed in TABLE 7. There was little difference between C. sapidus survival to megalopa and to 1st crab in 5% MAF and seawater control, but survival in 5% SPP was less than in the control. There was differential survival, however, from 5% MAF and SPP to 100% MAF and SPP.

Statistical Analysis of C. sapidus Survival in MAF

Statistical analysis idicated that:

(i) Survival to megalopa (TRFSZ) and to first crab (TRFSC) are both linearly related to % MAF (CONC) over the entire range 0-100%.

(ii) The two lines (Figure 1) are nearly parallel but statistical tests indicate a significant difference in the slopes (b values).

The summary equations are:

Zoeal : TRFSZ = 37.9 - 0.3585 * CONC

b = -0.3585 ± 0.0203 Degrees/% change in MAF

or = -3.585 ± 0.203 Degrees/10% change in MAF or approximately a 4% decrease in survival of zoea/10% increase in MAF (measured @ 50% CONC).

First Crab : TRFSC = 33.5 - 0.3303 * CONC.

b = -0.3303 ± 0.0660 Degrees/% change in MAF

or = -3.303 ± 0.660 Degrees/10% change in MAF or approximately 3% decrease in survival to first crab/10% increase in MAF (measured @ 50% CONC).

Statistical Analysis of C. sapidus in SPP

Statistical analysis indicated that:

(i) Survival to megalopa (TRFSZ) and to first crab (TRFSC) are both linearly related to % SPP (CONC) in the range 0-50%. There was no survival at the 100% CONC of SPP. Extrapolation estimates of total zoeal mortality at 75% SPP and of total first crab mortality at 70% SPP.

(ii) The two lines (Figure 2) are parallel since no significant difference was found between the two slopes. The single slope was estimated from the pooled data.

The summary equations are:

Zoeal : TRFSZ = 35.9 - 0.4722 * CONC.

First Crab : TRFSC = 33.1 - 0.4722 * CONC.

b = -0.4722 ± 0.1142 Degrees/% change in SPP

or = -4.722 ± 1.142 Degrees/10% change in SPP or approximately 5% decrease in survival (to either stage)/10% increase in SPP (measured @ 50% CONC).

Duration of C. sapidus in Larval Development

TABLE 6 gives the percent duration in days through zoeal and megalopa development as well as duration from hatch to 1st crab of each of three series (CsI-III) of Callinectes sapidus reared in seawater control and different concentrations of MAF and SPP. TABLE 7 lists the average duration in days of zoeal and megalopa development and duration from hatch to crab of the three

TABLE 2. PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF THREE SERIES (Rh I-III) OF Rhithropanopeus harrisii REARED IN SEAWATER CONTROL AND IN 4 CONCENTRATIONS OF MUD AQUEOUS FRACTION (MAF) AND 4 CONCENTRATIONS OF SUSPENDED PARTICULATE PHASE (SPP) OF USED LIGNOSULFONATE TYPE MUD (NO. 4 MUD).

Culture Media Salinity 20°/°° Temp. 25°C	Initial No. of larvae per series	% Survival to		Mean Duration of Development in days		
		Megalopa	1st crab	Zoea	Megalopa	Hatch to 1st Crab
Seawater Control	RhI-50	100	98	12.5	11.1	23.6
	RhII-50	92	90	10.9	6.7	17.6
	RhIII-50	96	94	12.2	6.0	18.2
5% MAF	RhI-50	96	94	12.5	10.8	23.3
	RhII-50	100	98	10.5	6.6	17.1
	RhIII-50	100	100	11.1	5.6	16.7
25% MAF	RhI-50	94	92	12.4	9.5	21.9
	RhII-50	92	92	10.6	6.0	16.6
	RhIII-50	96	96	12.0	5.6	17.6
50% MAF	RhI-50	94	92	12.4	8.7	21.1
	RhII-50	96	96	10.7	6.0	16.7
	RhIII-50	96	96	12.1	5.3	17.4
100% MAF	RhI-50	92	92	12.7	8.5	21.2
	RhII-50	94	90	10.9	5.5	16.4
	RhIII-50	90	90	12.3	5.3	17.6
5% SPP	RhI-50	96	96	12.4	10.9	23.3
	RhII-50	90	90	11.2	6.3	17.5
	RhIII-50	98	96	11.8	6.0	17.8
25% SPP	RhI-50	94	94	13.1	9.9	23.0
	RhII-50	96	96	11.1	6.6	17.7
	RhIII-50	96	96	11.8	5.6	17.4
50% SPP	RhI-50	96	94	12.7	9.5	22.2
	RhII-50	96	96	11.2	6.0	17.2
	RhIII-50	90	90	11.9	5.9	17.8
100% SPP	RhI-50	94	94	13.0	8.8	21.8
	RhII-50	96	96	11.5	6.2	17.7
	RhIII-50	96	96	11.9	5.6	17.5

TABLE 3. AVERAGE PERCENT SURVIVAL AND AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMENT OF THREE SERIES (Rh I-III) OF R. harrisii REARED IN SEAWATER CONTROL, 4 CONCENTRATIONS OF MAF AND 4 CONCENTRATIONS OF SPP OF USED LIGNOSULFONATE TYPE MUD (NO. 4 MUD).

Culture Media Salinity 20°/°° Temp. 25°C	Initial No. of larvae per series	% Survival to		Mean Duration of Development in days		
		Megalopa	1st crab	Zoea	Megalopa	Hatch to 1st Crab
Seawater Control	RhI-50 RhII-50 RhIII-50	96.0	94.0	11.9	7.9	19.8
5% MAF	RhI-50 RhII-50 RhIII-50	98.7	97.3	11.4	7.7	19.0
25% MAF	RhI-50 RhII-50 RhIII-50	94.0	93.3	11.7	7.0	18.7
50% MAF	RhI-50 RhII-50 RhIII-50	95.3	94.7	11.7	6.7	18.4
100% MAF	RhI-50 RhII-50 RhIII-50	92.0	90.7	11.9	6.4	18.4
5% SPP	RhI-50 RhII-50 RhIII-50	94.7	94.0	11.8	7.7	19.4
25% SPP	RhI-50 RhII-50 RhIII-50	95.3	95.3	12.0	7.4	19.4
50% SPP	RhI-50 RhII-50 RhIII-50	94.0	93.3	11.9	7.1	19.0
100% SPP	RhI-50 RhII-50 RhIII-50	95.3	95.3	12.1	6.9	19.0

TABLE 4. PERCENT MORTALITY IN DEVELOPMENTAL STAGES OF R. harrisii (Rh I-III) REARED IN SEAWATER CONTROL AND DIFFERENT CONCENTRATIONS OF MAF AND SPP OF USED LIGNOSULFONATE TYPE MUD (NO. 4 MUD).

Media	Series	Zoeal Stages I	II	III	IV	Megalopa	Total
Seawater Control	RhI-50	0	0	0	0	2	2
	RhII-50	2	4	2	0	2	10
	RhIII-50	0	2	0	2	2	6
5% MAF	RhI-50	2	2	0	0	2	6
	RhII-50	0	0	0	0	2	2
	RhIII-50	0	0	0	0	0	0
25% MAF	RhI-50	6	0	0	0	2	8
	RhII-50	4	2	2	0	0	8
	RhIII-50	2	0	2	0	0	4
50% MAF	RhI-50	4	2	0	0	2	8
	RhII-50	2	2	0	0	0	4
	RhIII-50	0	0	0	4	0	4
100% MAF	RhI-50	6	0	0	2	0	8
	RhII-50	0	2	0	4	4	10
	RhIII-50	2	0	0	8	0	10
5% SPP	RhI-50	4	0	0	0	0	4
	RhII-50	6	2	2	0	0	10
	RhIII-50	0	0	0	2	2	4
25% SPP	RhI-50	6	0	0	0	0	6
	RhII-50	0	2	2	0	0	4
	RhIII-50	2	0	0	2	0	4
50% SPP	RhI-50	4	0	0	0	2	6
	RhII-50	4	0	0	0	0	4
	RhIII-50	4	0	0	6	0	10
100% SPP	RhI-50	4	2	0	0	0	6
	RhII-50	2	0	2	0	0	4
	RhIII-50	0	0	0	4	0	4

TABLE 5. AVERAGE PERCENT MORTALITY IN DEVELOPMENTAL STAGES OF R. harrisii (Rh I-III) REARED IN SALTWATER CONTROL AND DIFFERENT CONCENTRATIONS OF MAF AND SPP OF USED LIGNOSULFONATE TYPE MUD.

Media	Series	Zoeal Stages I	II	III	IV	Megalopa	Total
Seawater Control	RhI-50 RhII-50 RhIII-50	0.7	2.0	0.7	0.7	2.0	6.0
5% MAF	RhI-50 RhII-50 RhIII-50	0.7	0.7	0	0	1.3	2.7
25% MAF	RhI-50 RhII-50 RhIII-50	4.0	0.7	1.3	0	0.7	6.7
50% MAF	RhI-50 RhII-50 RhIII-50	2.0	1.3	0	1.3	0.7	5.3
100% MAF	RhI-50 RhII-50 RhIII-50	2.7	0.7	0	4.7	1.3	9.3
5% SPP	RhI-50 RhII-50 RhIII-50	3.3	0.7	0.7	0.7	0.7	6.0
25% SPP	RhI-50 RhII-50 RhIII-50	2.7	0.7	0.7	0.7	0	4.7
50% SPP	RhI-50 RhII-50 RhIII-50	4.0	0	0	2.0	0.7	6.7
100% SPP	RhI-50 RhII-50 RhIII-50	2.0	0.7	0.7	1.3	0	4.7

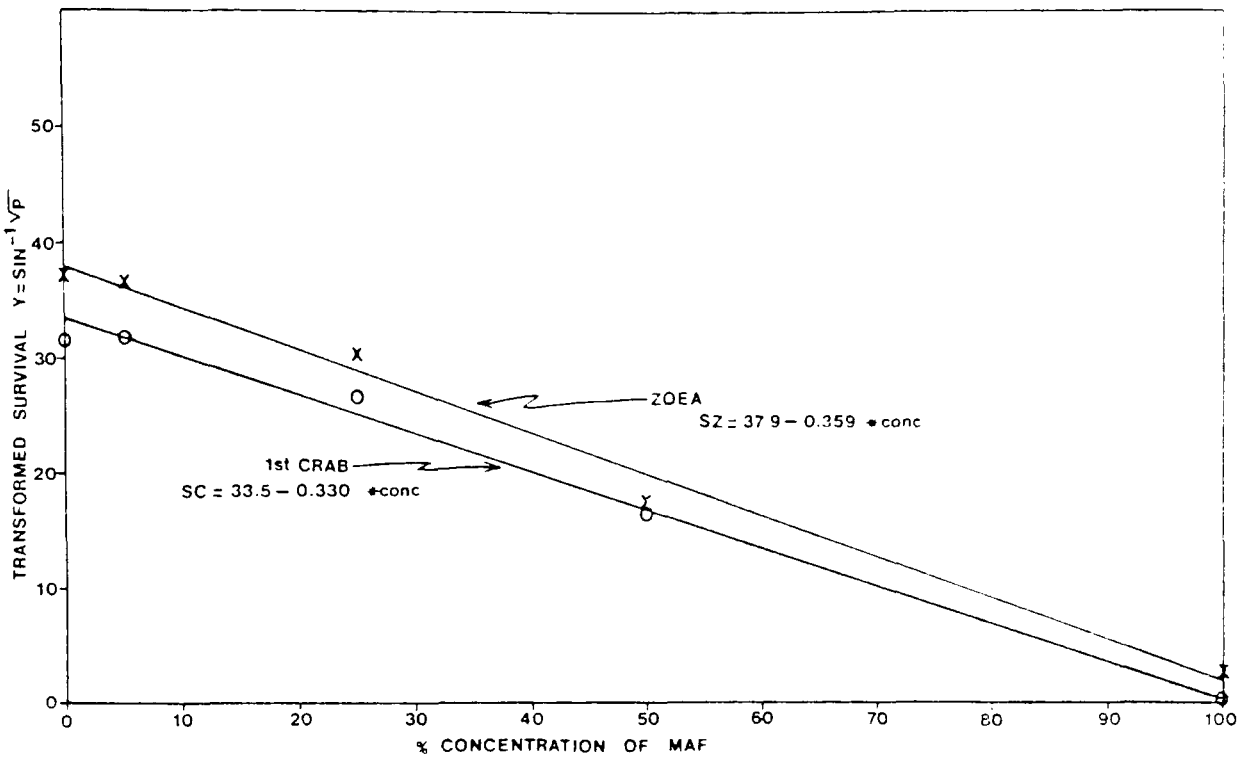

Figure 1. Effect of percent of mud aqueous fraction (MAF) of used light weight lignosulfonate type mud on survival of C. sapidus larvae.
Hatch to megalopa x——— x
Hatch to 1st crab o——— o

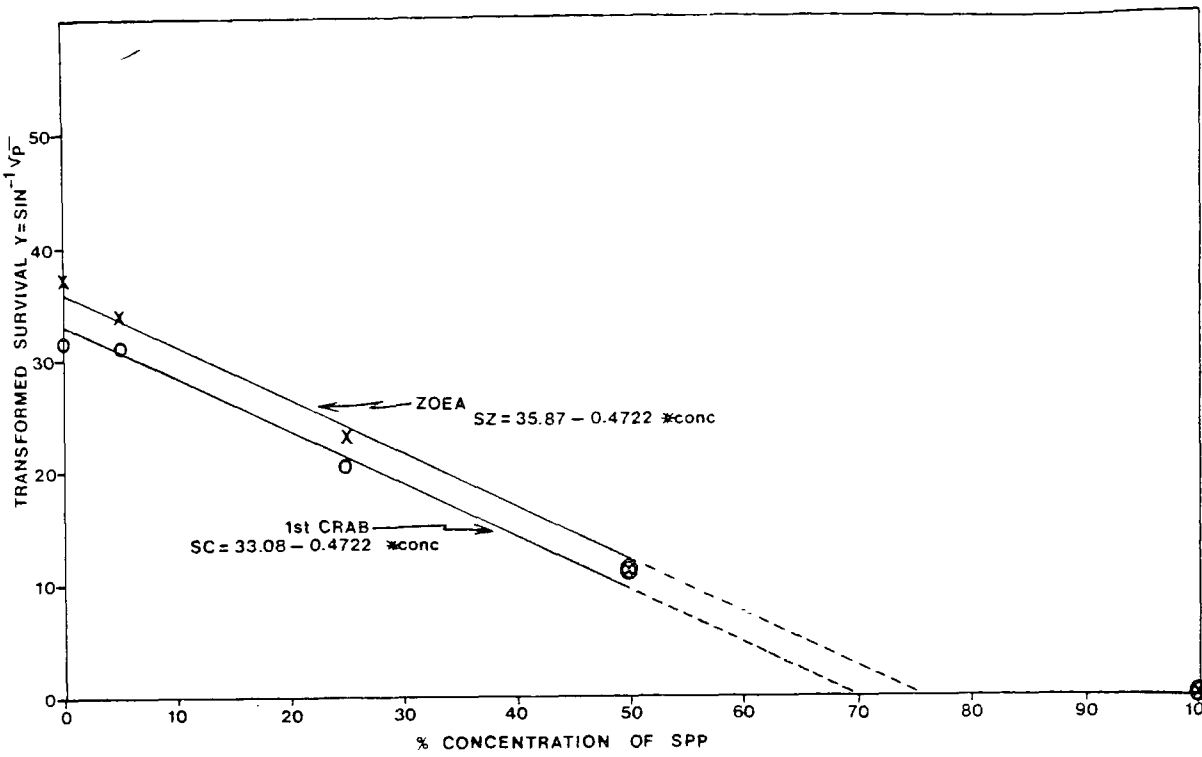

Figure 2. Effect of percent of suspended particulate phase (SPP) of used light weight lignosulfonate type mud on survival of C. sapidus larvae.
Hatch to megalopa x—— x
Hatch to 1st crab o—— o

series of C. sapidus reared in seawater control and in four concentrations of MAF and SPP. There is no significant difference in duration in zoeal development and in hatch to crab in seawater control and the concentrations of MAF and SPP employed.

Mortality of C. sapidus by Larval Stage

Callinectes sapidus may pass through seven, occasionally eight, zoeal stages before it molts into a megalopa, a ninth stage of development. In an effort to determine if larvae in one or more of the nine developmental stages of C. sapidus were particularly sensitive to different concentrations of MAF and SPP, a record of deaths by stages was made for larvae from each of three crabs, CsI-III, which had been reared in seawater control and four concentrations of MAF and SPP (TABLE 8). Average percent mortality of larvae in developmental stages of C. sapidus of all series reared in seawater control and different concentrations of MAF and SPP is given in TABLE 9.

Statistical Analysis of C. sapidus Cumulative Mortality by Stages When Reared in MAF

The results illustrated in Figure 3 show the effect of MAF concentration on the mortality of larvae at each stage of development. The percent mortalities on the graph were obtained from the means of the transformed variable. Mortality of larvae in 5 and 25% MAF was not significantly different from mortality in the control in any of the nine developmental stages, but mortality of larvae reared in 50 and 100% MAF was significantly different from the control in every developmental stage. Although larvae in zoeal stage I were most sensitive, but larvae in zoeal stage II were also very sensitive, for significant increases in mortality over the previous stage occurred in this stage in all media (Figure 3).

Statistical Analysis of C. sapidus Cumulative Mortality by Stages When Reared in SPP

Mortality of larvae in 5% SPP was not significantly different from mortality in the control in any of the nine developmental stages, but mortality of larvae reared in 50 and 100% SPP was significantly different from the control in every developmental stage. In zoeal stage I 25% SPP was significantly different from the control at the 0.05 level. As in the MAF experiment, larvae in zoeal stage I were most sensitive (Figure 4), but larvae in zoeal stage II were also very sensitive, for significant increases in mortality over the previous stage occurred in zoeal stage II in all media (Figure 4).

Larval Behavior: Swimming of C. sapidus Larvae Upon Exposure to Drilling Mud MAF and SPP

Blue crab larval behavior is affected by exposure to MAF and SPP with the general effect being a decline in swimming speed (Table 10). A significant reduction in speed is only observed in the 100% solution of MAF. However, all solutions of SPP cause a significant decline. The highest two concentrations cause the greatest reduction.

TABLE 6. PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF THREE SERIES (Cs I-III) OF Callinectes sapidus REARED IN SEAWATER CONTROL AND IN DIFFERENT CONCENTRATIONS OF MAF AND SPP OF USED LIGNOSULFONATE TYPE MUD

Culture Media Salinity 30°/°° Temp. 25°C	Initial No. of larvae per series	% Survival to		Mean Duration of Development in days		
		Megalopa	1st crab	Zoea	Megalopa	Hatch to 1st Crab
Seawater Control	CsI-50	70	50	30.0	7.5	37.2
	CsII-50	30	22	41.9	8.0	49.7
	CsIII-50	14	14	32.8	7.8	40.7
5% MAF	CsI-50	66	54	32.3	8.2	40.7
	CsII-50	36	32	38.9	7.2	45.1
	CsIII-50	10	6	33.2	6.5	39.8
25% MAF	CsI-50	48	42	32.3	7.7	39.7
	CsII-50	24	20	35.0	6.4	44.5
	CsIII-50	10	8	37.0	7.3	44.5
50% MAF	CsI-50	4	4	34.0	6.0	40.0
	CsII-50	18	14	39.9	7.4	47.4
	CsIII-50	8	8	33.8	6.5	40.3
100% MAF	CsI-50	2	0	34.0	-	-
	CsII-50	0	0	0	-	-
	CsIII-50	0	0	-	-	-
5% SPP	CsI-50	46	40	32.4	7.5	39.5
	CsII-50	32	26	34.1	6.8	44.3
	CsIII-50	18	16	32.1	7.7	40.0
25% SPP	CsI-50	14	10	34.1	7.0	41.2
	CsII-50	26	20	41.5	7.8	49.9
	CsIII-50	8	8	33.0	7.0	40.0
50% SPP	CsI-50	2	2	32.0	7.0	39.0
	CsII-50	4	4	39.0	9.0	48.0
	CsIII-50	6	6	36.3	7.3	43.7
100% SPP	CsI-50	0	0	-	-	-
	CsII-50	0	0	-	-	-
	CsIII-50	0	0	-		-

TABLE 7. AVERAGE PERCENT SURVIVAL AND AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMENT OF THREE SERIES (Cs I-III) OF *C. sapidus* IN SEAWATER CONTROL AND DIFFERENT CONCENTRATIONS OF MAF AND SPP OF USED LIGNOSULFONATE TYPE MUD.

Culture Media Salinity 30°/°° Temp. 25°C	Initial No. of larvae per series	% Survival to		Mean Duration of Development in days		
		Megalopa	1st crab	Zoea	Megalopa	Hatch to 1st Crab
Seawater Control	CsI-50 CsII-50 CsIII-50	37.3	28.0	34.9	7.8	42.5
5% MAF	CsI-50 CsII-50 CsIII-50	38.0	31.3	34.8	7.3	41.9
25% MAF	CsI-50 CsII-50 CsIII-50	27.3	23.3	34.8	7.1	42.9
50% MAF	CsI-50 CsII-50 CsIII-50	10.0	8.7	35.9	6.6	42.6
100% MAF	CsI-50 CsII-50 CsIII-50	0.7	-	-		-
5% SPP	CsI-50 CsII-50 CsIII-50	31.3	26.7	32.4	7.3	41.3
25% SPP	CsI-50 CsII-50 CsIII-50	15.3	12.0	36.2	7.3	43.7
50% SPP	CsI-50 CsII-50 CsIII-50	4.0	4.0	35.8	7.8	43.6
100% SPP	CsI-50 CsII-50 CsIII-50	0	0	-	-	-

TABLE 8. PERCENT MORTALITY IN DEVELOPMENTAL STAGES OF THREE SERIES (Cs I-III) OF C. sapidus REARED IN SALTWATER CONTROL AND DIFFERENT CONCENTRATIONS OF MAF AND SPP OF USED LIGNOSULFONATE TYPE MUD.

		Zoeal Stages									
Media	Series	I	II	III	IV	V	VI	VII	VIII	Megalopa	Total
Seawater Control	CsI-50	16	8	2	0	0	0	2	2	20	50
	CsII-50	28	28	12	2	0	0	0	0	8	78
	CsIII-50	32	42	8	4	0	0	0	0	0	86
5% MAF	CsI-50	28	4	0	0	0	0	0	2	12	46
	CsII-50	18	24	8	8	2	2	2	0	4	68
	CsIII-50	54	32	2	0	0	0	2	0	4	94
25% MAF	CsI-50	42	8	0	2	0	0	0	0	6	58
	CsII-50	36	26	6	2	0	2	2	2	4	80
	CsIII-50	32	42	10	6	0	0	0	0	2	92
50% MAF	CsI-50	74	12	8	0	0	0	0	2	0	96
	CsII-50	58	18	4	2	0	0	0	0	4	86
	CsIII-50	74	12	2	0	0	0	2	2	0	92
100% MAF	CsI-50	98	0	0	0	0	0	0	0	2	100
	CsII-50	90	10	0	0	0	0	0	0	0	100
	CsIII-50	96	4	0	0	0	0	0	0	0	100
5% SPP	CsI-50	30	14	4	2	0	0	0	4	6	60
	CsII-50	34	26	2	0	0	0	2	4	6	74
	CsIII-50	62	16	2	0	0	2	0	0	2	84
25% SPP	CsI-50	60	24	2	0	0	0	0	0	4	90
	CsII-50	46	18	8	2	0	0	0	0	6	80
	CsIII-50	52	20	14	2	0	0	0	4	0	92
50% SPP	CsI-50	94	2	0	2	0	0	0	0	0	98
	CsII-50	74	20	2	0	0	0	0	0	0	96
	CsIII-50	60	26	6	0	0	0	2	0	0	94
100% SPP	CsI-50	100	0	0	0	0	0	0	0	0	100
	CsII-50	88	12	0	0	0	0	0	0	0	100
	CsIII-50	92	6	2	0	0	0	0	0	0	100

TABLE 9. AVERAGE PERCENT MORTALITY IN DEVELOPMENTAL STAGES OF THREE SERIES (Cs I-III) OF *C. sapidus* REARED IN SALTWATER CONTROL AND DIFFERENT CONCENTRATIONS OF MAF AND SPP OF USED LIGNOSULFONATE TYPE MUD.

Media	Series	Zoeal Stages I	II	III	IV	V	VI	VII	VIII	Megalopa	Total
Seawater Control	CsI-50 CsII-50 CsIII-50	25.3	26.0	6.7	2.0	0	0	0.7	0.7	9.3	71.3
5% MAF	CsI-50 CsII-50 CsIII-50	33.3	20.0	3.3	2.7	0.7	0.7	1.3	0.7	6.7	69.4
25% MAF	CsI-50 CsII-50 CsIII-50	36.7	25.3	5.3	3.3	0	0.7	0.7	0.7	4.0	76.7
50% MAF	CsI-50 CsII-50 CsIII-50	68.7	14.0	4.7	0.7	0	0	0.7	1.3	1.3	91.3
100% MAF	CsI-50 CsIII-50 CsIII-50	94.7	4.7	0	0	0	0	0	0	0.7	100
5% SPP	CsI-50 CsII-50 CsIII-50	42.0	18.7	2.7	0.7	0	0.7	0.7	2.7	4.7	72.7
25% SPP	CsI-50 CsII-50 CsIII-50	52.7	20.7	8.0	1.3	0	0	0	1.3	3.3	87.3
50% SPP	CsI-50 CsII-50 CsIII-50	76.0	16.0	2.7	0.7	0	0	0.7	0	0	96
100% SPP	CsI-50 CsII-50 CsIII-50	93.3	6.7	6.7	0	0	0	0	0	0	100

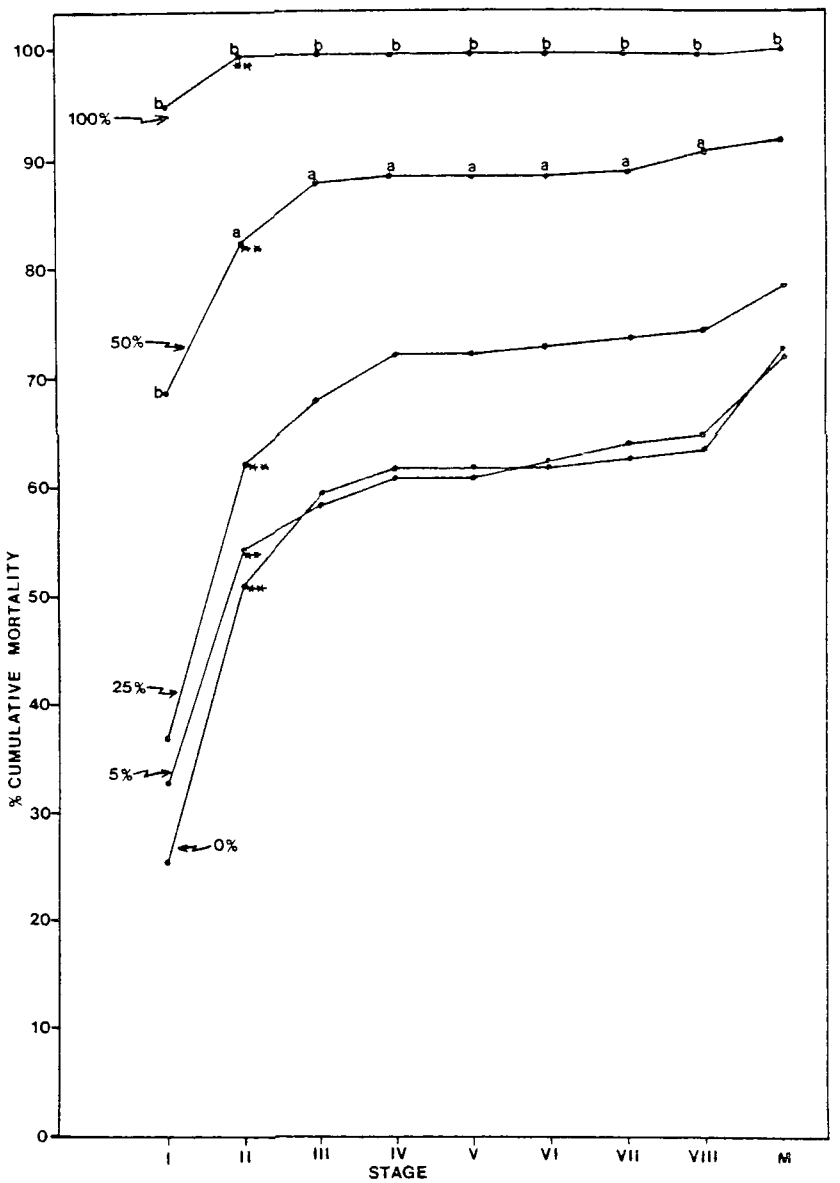

Figure 3. Effect of MAF of used light-weight lignosulfonate type mud on mortality by stages of development of C. sapidus.
a. Significantly different from control (0.05)
b. Significantly different from control (0.01)
*. Significant increase over previous stage (0.05)
**. Significant increase over previous stage (0.01)

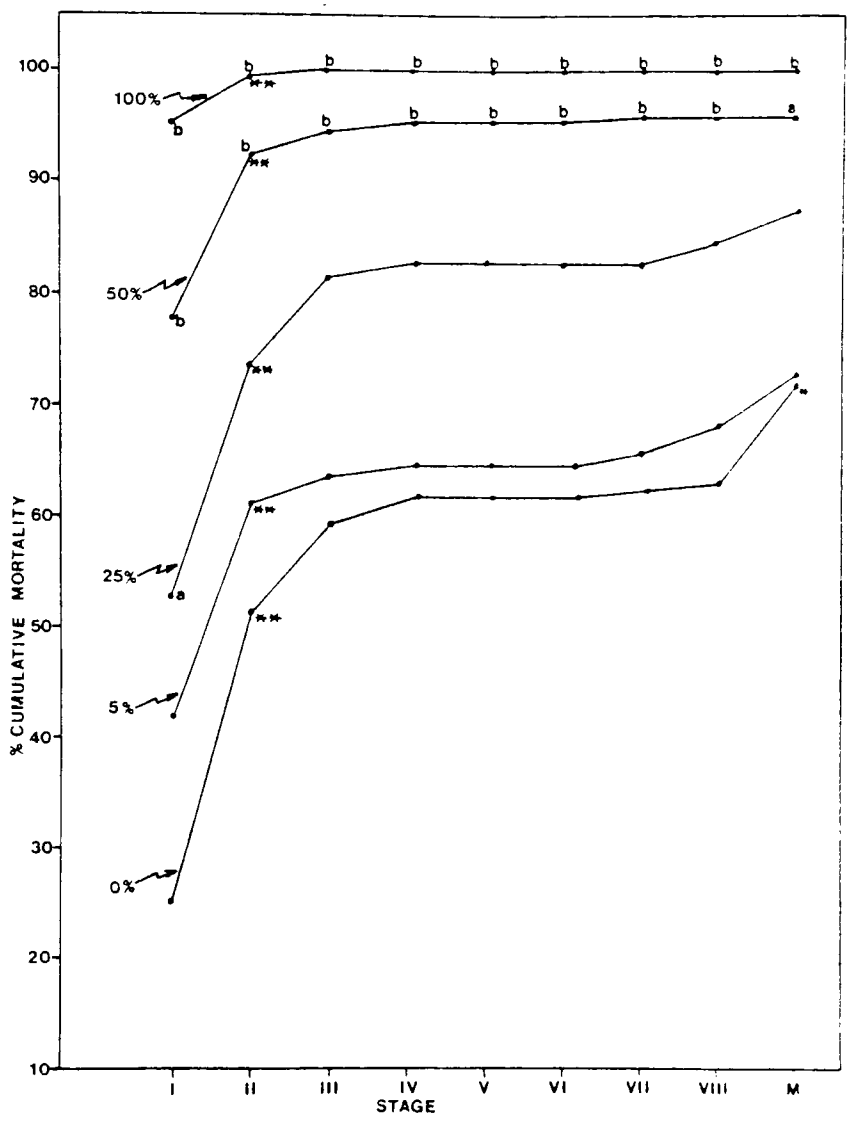

Figure 4. Effect of SPP of used light-weight lignosulfonate type mud on mortality by stages of development of C. sapidus.
a. Significantly different from control (0.05)
b. Significantly different from control (0.01)
*. Significant increase over previous stage (0.05)
**. Significant increase over previous stage (0.01)

TABLE 10. SWIMMING SPEED (mm/min) OF CONTROL C. sapidus FIRST ZOEAE AND THOSE TREATED WITH DIFFERENT CONCENTRATIONS OF MAF AND SPP. THE MEAN (M) AND STANDARD DEVIATION (SD) ARE SHOWN. THE SAMPLE SIZE FOR EACH CONDITION IS 60. * INDICATES $P < 0.02$ STATISTICAL DIFFERENCE BETWEEN CONTROL LARVAE AND EXPOSURE TO A PARTICULAR CONCENTRATION, WHILE ** IS $P < 0.01$ AND *** $P < 0.001$.

Condition	M	SD
control	100.2	28.9
5% MAF	94.6	33.2
25% MAF	94.9	36.7
50% MAF	89.6	39.5
100% MAF	60.7**	24.7
5% SPP	81.9**	35.0
25% SPP	86.0*	31.6
50% SPP	60.2***	24.3
100% SPP	61.2***	25.3

Survival

Mud aqueous fractions (MAF) and suspended particulate phase (SPP) of used low density lignosulfonate type drilling fluid were nontoxic to the development of Rhithropanopeus harrisii from the time of hatching to the 1st crab stage. TABLES 2 and 3 reveal that survival was 90% percent or greater in seawater control and in concentrations of 5 to 100% MAF and SPP. In a similar experiment using Callinectes sapidus, there was differential survival from hatching to megalopa and to 1st crab stage in concentrations of 5 to 100% MAF and SPP (TABLES 6 and 7; Figures 1 and 2). The sensitivity of R. harrisii and C. sapidus larvae to a pollutant, such as mirex, may be similar (Bookhout et al., 1972; Bookhout and Costlow, 1975), or very different; sublethal concentrations of Kepone to R. harrisii larvae ranged from 35 to 80 ppb, whereas the sublethal concentrations to C. sapidus larvae ranged from 0.1 to 1.0 ppb (Bookhout et al., 1980). We do not know why R. harrisii are so much more resistant to Kepone and MAF and SPP of low density lignosulfonate.

In chronic toxicity studies of the larval development of crabs, sublethal concentrations of a pollutant are arbitrarily defined as those in which there is a reduction in survial with increased concentration of the pollutant and in which at least 10% reach the 1st crab stage. Acutely toxic concentrations are those in which less than 10% of the larvae reach the 1st crab stage (Epifanio, 1971; Bookhout and Costlow, 1975). Since survival to the 1st crab stage in 5% MAF was somewhat better than in seawater control, 5% is considered nontoxic. Twenty-five percent MAF, 5 and 25% SPP are sublethal, and concentrations of 50 and 100% MAF and SPP are acutely toxic.

Survival to 1st crab was better in MAF than in SPP. In 5% (5,000 ppm), 25% (25,000 ppm), 50% (50,000 ppm) and 100% (100,000 ppm) MAF survival was 31.3, 23.3, and 8.7% and 0 respectively, whereas in 5% (5,000 ppm), 25% (25,000 ppm), 50% (50,000) ppm and 100% (100,000 ppm) SPP, it was 26.7, 12.0, and 4.0% and 0 respectively (TABLES 1 and 2).

Behavior

Blue crab larval behavior was affected by exposure to MAF and SPP with the general effect being a decline in swimming speed (TABLE 10). A significant reduction in speed was only observed in 100% MAF, whereas all solutions of SPP caused a significant decline with the highest two concentrations causing the greatest reduction. Carls and Rice (1981), in a similar study of stage I crab and shrimp zoeae exposed to fractions of used drilling fluids from Alaska, reported that behavioral observations were a more sensitive indicator of mud toxicity than mortality. The effective concentrations (EC50 at 144 h), as measured by the cessation of swimming, could be determined at lower concentrations than LC50 at 144 h. Thus, we are of the opinion that a significant change in the rate of swimming indicates sublethal stress.

Chronic vs Acute Toxicity Tests

As far as known, no chronic studies have been made on the effect of MAF and SPP of whole drilling fluids on the complete larval development of any marine organism. In this investigation, it was used because, in our opinion, a test covering the entire larval development of crabs would give a better evaluation of the possible toxicity of drilling fluids in the field than acute toxicity study of 96 h. A chronic toxicity test would include all periods of molting when crustacean larvae are known to be very sensitive to toxic substances. Furthermore, if records are kept of mortality at each stage of development, it is possible to determine which stage or stages in the larval period are particularly sensitive (Figures 3 and 4). An acute toxicity test of 96 h would not have the advantages given for chronic tests and would not include the particuarly sensitive 1st molt in zoeal development of a blue crab (Bookhout and Costlow, 1975).

Numerous acute toxicity studies have been made on the effects of MAF and SPP of whole drilling fluids on adult marine organisms and some have been done on individual larval stages. In these tests, the median lethal concentration, LC50, is defined as that concentration lethal to 50% of the test organism within a specified test period, usually 96 h. In this study, the results of acute toxicity studies (96-h LC50) could not be compared directly with the results of the long term chronic studies because less than 50% of C. sapidus survived in seawater control. Generalizations may be made, however, from both types of studies.

There is sufficient evidence in the literature to conclude that adult marine invertebrates are generally not affected by any type of drilling fluid but juveniles and larvae are. In a comparative study of spud mud and three types of lignosulfonate muds of different densities, Neff et al. (1980) reported that used spud mud was nontoxic to all larval and adult organisms tested. This mud is the type used near the surface and contains aqueous solutions of bentonite clay and some barite which are considered nontoxic. Aqueous extracts of the three other lignosulfonate drilling fluids are similar in their acute toxicity to larvae and juvenile crustaceans and were generally nontoxic to adult marine organisms. Ninety-six hour LC50 for 1st zoeae of grass shrimp, Palaemonetes pugio ranged from 18 to 35% (18,000 to 35,000 ppm) MAF of three types of lignosulfonate mud. The acute toxicity measured as 96 h LC50 of SPP of used high density lignosulfonate of 1 day (1st zoeae), 5 days (3rd zoeae) and 10 days (4th and 5th zoeae) was 11.8, 13.2 and 11.7% respectively. Thus SPP of high-weight lignosulfonate was more toxic than MAF of chrome lignosulfonate, mid-weight and high-weight lignosulfonate, as found in this investigation when C. sapidus larvae were exposed to similar fractions of used light-weight lignosulfonate.

Carr et al. (1980) found that the 96 h LC50 values for one-day old oppossum shrimp, Mysidopsis almyra, exposed to MAF of used chrome lignosulfonate drilling fluid which was static but replaced daily was 27% (27,000 ppm), as contrasted to 42% (42,000 ppm) when the culture was static but not changed in 96 hours. They believed the difference indicates that some toxic volatile components were lost during static exposure. They also found that as mysids aged from one to 14 days their tolerance to MAF of used chrome

lignosulfonate increased. In this investigation, as shown in TABLES 4 and 5 and Figures 3 and 4, the most sensitive zoeae of C. sapidus, as revealed by percent mortality, were in stages I and II. In the remainining zoeal stages, there was less mortality indicating that larvae became more tolerant in later stages, or that the more susceptible portion of the population was eliminated first.

Gerber et al. (1980) studied the effects of five types of used drilling fluids on 13 species of marine animals from the coastal Gulf of Maine waters. Except for spud mud, they were surprised to find that there was little difference in the toxicity of the other four muds since they contain different amounts of toxic substances. As in other investigations they found adult organisms exhibited little or no mortality to the highest concentrations of MAF drilling fluids. By contrast the acute toxicity measured as 96 h LC50 MAF of used light-density lignosulfonate drilling fluid of stage V zoeae of the American lobster, Homarus americanus, was 5% (5,000 ppm). Zoeal stage I of the northern shrimp, Pandalus borealis, exposed to used medium-density lignosulfonate had a 96-h LC50 of MAF and filtered MAF of 17 and 19% respectively, whereas in high-density lignosulfonate the 96-h LC50 of MAF and filtered MAF was 65 and 55%, respectively.

Environmental Implications

To determine the possible hazards of MAF and SPP of lignosulfonate on test larvae, such as those of Callinectes sapidus, it is necessary to know the extent of dispersion of the upper of two turbidity plumes from the point of discharge in relation to the rate and volume of discharge. MAF and SPP are fractions of whole mud that are incorporated in the upper turbidity plume. To correlate laboratory findings to field conditions, it is important to ascertain the extent of dispersion of the highest concentrations around the point of discharge and the gradient of reduction during dispersion peripherally until the area of background level is reached.

Ray and Meek (1980) studied eight discharge plumes for 85 days from an offshore exploratory well on Tanner Bank. Six resulted from mud discharges of 10 to 754 barrels per hour and the remaining two resulted from cutting discharges. There was an average initial dilution of 500-1000:1 of total suspended solids from lightly treated seawater lignosulfonate mud plumes within the first 3 m from the point of discharge. An additional 100:1 dilution occurred within the next 100 m and at 100 m suspended solids were reduced to background levels of 1.0 mg/l. Metal concentrations reached background levels at 100 to 150 m from the point of discharge. Gerber et al. (1980) estimated if within one to three meters of the discharge source mud components were diluted from 500:1 to 1000:1 under discharge conditions of 10 to 15 bbl/h, 100% MAF (100,000 ppm) and 5% MAF (5,000 ppm) would persist within a couple of meters from the discharge pipe. Occasionally high mud discharge rates might occur for less than an hour. Ayers et al. (1980) reported that under high discharge rates of 275 to 1000 bbl/h background levels of suspended solids in the upper turbidity plume were reached 600 to 1500 m from the source. Under short periods, 5% (5,000 ppm) MAF might represent conditions 20 to 35 m from the source. Petrazzuolo (1981 draft unpublished) reported that for almost all of

the species and fluids tested to date, acute lethal effects of drilling fluids would not be expected further than 15 m from one discharge.

If drilling operations were taking place in the shallow shelf waters off the southeast coast of United States or the coast of Gulf of Mexico, crab larvae probably would be in the vicinity of operations from late spring to early fall. Ovigerous blue crabs, Callinectes sapidus, are known to migrate from estuaries to the mouths of rivers and beyond before they shed approximately 2,500,000 zoeae per crab. Their larvae would become a part of the plankton and would be distributed by currents of shallow shelf waters off the southeast coast of the United States and Gulf of Mexico. Other crabs which belong to the same subfamily (Portuninae) as the commercial blue crab, such as other species of Callinectes, Portunus and Araeneus, also shed their larvae off the southeast and Gulf states for this is where the adults live. Although these larvae could be in the vicinity of drilling operations and might be found in the upper turbidity plume, the chances of many of the larvae remaining in the 3 m highly toxic zone or even in the 15 m intermediate toxic zone around the discharge source long enough to suffer mortality are very remote. If by chance a few 1st or 2nd stage zoeae of C. sapidus in the process of molting happened to be entrained in the near zone area of discharge, they might be killed or receive an irreversible stress, for in this investigation it was found that zoeae in stage I and II were very sensitive, especially at the time of molting.

SECTION 5

EFFECTS OF HEXAVALENT CHROMIUM ON THE COMPLETE LARVAL DEVELOPMENT OF CRABS, *Rhithropanopeus harrisii* AND *Callinectes sapidus*

INTRODUCTION

One of the trace metals in drilling fluids which may have a detrimental environmental effect is chromium. The toxicity of chromium to marine organisms will vary with valence state, pH and oxidation states. Hexavalent chromium (Cr^{+6}) is stable in seawater. It often appears as a soluble chromate or dichromate, powerful oxidants which can readily penetrate biological membranes and irritate cells (Mertz, 1969). Hexavalent chromium, as chromic oxide, chromate or dichromate, reacts with organic matter in acidic solution, leading to the trivalent form (Cr^{+3}). The trivalent form is associated chiefly with particulate matter, such as clay, which suggests that organic particulate matter may reduce and bind the hexavalent form in solution [National Academy of Science (NAS), 1974; Curl *et al.*, 1965]. Hexavalent chromium is much more toxic to organisms than trivalent chromium, in part because hexavalent chromium is water soluble and trivalent chromium has a very low solubility in seawater.

Chromium is contributed to drilling fluids chiefly by lignosulfonate which is added in greater amounts as mud weight is increased (Hrudey and Eng, 1979). Ferrochrome lignosulfonate, brand name "Q-Broxin," and chrome lignosulfonate are common additives to drilling fluids which contribute to Cr enrichment. Liss *et al.* (1980) reported that Q-Broxin included a metallic composition of 7% Na, 3% Cr, 1% Fe and 0.3% Ca W/W. Initially, Q-Broxin contains hexavalent chromate salts, but at temperatures between 120 to 175°C hexavalent chromium is converted to the trivalent state. The thinning property of Q-Broxin can be restored at temperatures between 120° and 175°C by adding more C^{+6}. Above 175°C no more C^{+6} will restore lost thinning ability.

Chrome lignosulfonate, containing hexavalent salts, is added to drilling fluids to improve their thermal stability and for corrosion protection. Carr *et al.* (1980) reviewed pertinent literature in reference to chrome lignosulfonate, including a Master's thesis by Knox (1978). According to Knox (1978) the lignosulfonate is attached to clay by being adsorbed to metals through phenolic oxygens, sulfonate groups and carboxilic acid groups. The rate of adsorption and the conversion of Cr^{+6} to Cr^{+3} is slow at room temperature, but rapid at high temperature. Additional chromate salts (Cr^{+6}) are often added to drilling fluids to further improve their thermal stability and corrosion protection. After drilling fluids have been used for an extended period of time, it is very probable that most of the chromium is associated with the clay fraction and the chromium is trivalent.

Knox (1978) has suggested that after drilling fluids are discharged into the ocean, chromium and associated material are released slowly in soluble form from clay particles into the water. Once freed from clay particles, Cr^{+3} through slow oxidation may revert to Cr^{+6} as Cranston and Murray (1980) discovered in their experiments. In other research, Cr^{+3} oxidation rates of 3% in 30 days occurred at 22 to 26°C, and at 35°C and 45°C the same extent of oxidation occurred at 10 days and less than 3 days, respectively. Fukai and Vas (1969) reported Cr^{+3} oxidation rates of 7% Cr^{+6} in one week. Most investigators assume that all of the chromium in drilling fluids is trivalent even though analyses were not made to determine the valence. Other investigators, as Liss et al. (1980), apparently are not certain that all of the chromium in drilling fluids is trivalent. Personal communication with several investigators concerning the presence of hexavalent chromium in discharged drilling fluids brought forth comments such as: "doubtful", "under certain conditions", "might vary from 5 to 20% depending upon input into drilling fluid and time sample was taken."

In used chrome lignosulfonate drilling mud taken from an offshore well after 20 days of drilling at a depth of 3,650 meters (12,000 feet), the whole mud contained approximately 500 ppm total chromium on a dry weight basis (Neff et al., 1981). The mud filtrate contained 27 ppm total chromium and the mud aqueous fraction had less than 1 ppm total chromium. Carr et al. (1981) reported that in a preliminary analysis more than 75% of the chromium was found in a trivalent state.

We can conclude from the above discussion that the possibility exists that under certain conditions both trivalent and hexavalent chromium may be discharged. Most of the discharge would undoubtedly include trivalent chromium and not be too bioavailable for planktonic organisms.

If hexavalent chromium were included in the discharge, it would be more bioavailable than trivalent chromium and in the course of time would bioaccumulate if assimilated. Mearns et al. (1976) and other investigators have shown that hexavalent chromium is many times more toxic than trivalent chromium.

This investigation was undertaken to determine the concentrations of hexavalent chromium, Na_2CrO_4, which are nontoxic, sublethal and acutely toxic to the complete larval development of the mud crab, Rhithropanopeus harrisii and the blue crab, Callinectes sapidus.

Survival of Rhithropanopeus harrisii Larvae

The percent survival of R. harrisii from hatching to megalopa and to 1st crab is given for each replicate series in TABLE 11. The average percent survival of all R. harrisii series reared in seawater control and four concentrations of Na_2CrO_4 is given in TABLE 12. There are no significant differences between survival to megalopa and to 1st crab stage reared in seawater control and 1 ppm (TABLE 12). There was a decrease in survival with an increase in concentration from 1 to 29 ppm Na_2CrO_4.

Statistical Analysis of R. harrisii Larval Survival

Statistical analysis indicated that:

(i) survival to megalopa (TRFSZ) is linearly related to concentration of Na_2CrO_4 (CONC) in the range 0-40 ppm. Survival to first crab (TRFSC) is linearly related to concentration of Na_2CrO_4 (CONC) in the range 0-29 ppm.

(ii) the two lines (Figure 5) are not parallel since the two slopes differ significantly.

The summary equations are:

Zoea: TRFSZ = 79.5 - 1.936 * CONC.

b = -1.936 ± 0.088 Degrees/ppm CONC,

or = -19.36 ± 0.88 Degrees/10 ppm CONC,
or approximately 34% decrease in survival of zoea/10 ppm increase in Na_2CrO_4 (measured @ 50% survival).

First Crab: TRFSC = 76.1 - 2.274 * CONC.

b = -2.274 ± 0.088 Degrees/ppm CONC,

or = -22.74 ± 0.88 Degrees/10 ppm CONC,
or approximately 40% decrease in survival to first crab/10 ppm increase in Na_2CrO_4 (measured @ 50% survival).

Estimated LC50 values were obtained by setting each equation equal to 45 degrees (50% survival) and solving each equation for the value of CONC.

Estimated LC50 values were:

Zoea: 17.8 ppm Na_2CrO_4

First Crab: 13.7 ppm Na_2CrO_4

TABLE 11. PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *Rhithropanopeus harrisii* REARED IN SEAWATER CONTROL AND IN DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM, Na_2CrO_4.

Culture Media Salinity 20°/°° Temp. 25°C	Initial No. of larvae per series	% Survival to		Mean Duration of Development in days		
		Megalopa	1st crab	Zoea	Megalopa	Hatch to 1st Crab
Seawater Control	RhI-50	96	96	12.4	8.6	21.0
	RhII-50	90	88	13.0	6.1	19.2
	RhIII-50	96	92	10.6	6.7	17.3
	RhIV -50	94	94	11.8	7.9	21.7
	RhV-50	98	96	11.8	5.4	17.2
	RhVI-50	96	96	11.7	5.6	17.3
1.12 ppm Na_2CrO_4	RhI-50	94	94	12.4	9.1	21.5
	RhII-50	90	90	13.0	6.1	19.1
	RhIII-50	90	86	11.8	6.2	18.0
	RhIV-50	98	96	11.8	7.0	19.4
	RhV-50	98	98	11.8	5.4	17.2
	RhVI-50	98	98	11.9	5.5	17.4
7.17 ppm Na_2CrO_4	RhI-50	90	90	12.7	7.5	20.2
	RhII-50	72	38	14.2	6.2	20.3
	RhIII-50	84	62	12.1	6.0	18.1
	RhIV-50	80	56	12.3	6.9	18.9
	RhV-50	94	92	12.3	5.5	18.0
	RhVI-50	80	74	12.6	5.6	18.4
14.52 ppm Na_2CrO_4	RhI-50	98	82	13.7	6.7	20.5
	RhII-50	56	18	14.7	6.6	20.9
	RhIII-50	58	48	12.6	6.0	18.5
	RhIV-50	18	8	12.8	6.3	18.9
	RhV-50	84	76	13.6	5.4	19.1
	RhVI-50	62	52	14.3	7.1	21.0
29.09 ppm Na_2CrO_4	RhI-50	60	14	15.4	6.3	21.4
	RhII-50	4	0	15.5	-	-
	RhIII-50	0	0	-	-	-
	RhIV-50	0	0	-		-
	RhV-50	34	6	15.9	8.0	23.0
	RhVI-50	34	22	14.9	7.8	22.8
40.60 ppm Na_2CrO_4	RhIII-50--RhVI-50	0	0	-	-	-
46.40 ppm Na_2CrO_4	RhI,II,V,VI-50	0	0	-	-	-
58.09 ppm Na_2CrO_4	RhI,II,V,VI-50	0	0	-	-	-

TABLE 12. AVERAGE PERCENT SURVIVAL AND AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMENT OF *R. harrisii* IN SEAWATER CONTROL AND DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM, Na_2CrO_4.

Culture Media Salinity 20°/°° Temp. 25°C	Initial No. of larvae per series	% Survival to		Mean Duration of Development in days		
		Megalopa	1st crab	Zoea	Megalopa	Hatch to Crab
Seawater Control	RhI-50 RhII-50 RhIII-50 RhIV-50 RhV-50 RhVI-50	95.0	93.7	11.9	6.7	19.0
1.12 ppm Na_2CrO_4	RhI-50 RhII-50 RhIII-50 RhIV-50 RhV-50 RhVI-50	94.7	93.7	12.1	6.6	18.8
7.17 ppm Na_2CrO_4	RhI-50 RhII-50 RhIII-50 RhIV-50 RhV-50 RhVI-50	83.3	68.7	12.7	6.3	19.0
14.52 ppm Na_2CrO_4	RhI-50 RhII-50 RhIII-50 RhIV-50 RhV-50 RhVI-50	62.7	47.3	13.6	6.4	19.8
29.09 ppm Na_2CrO_4	RhI-50 RhII-50 RhIII-50 RhIV-50 RhV-50 RhVI-50	22.0	7.0			

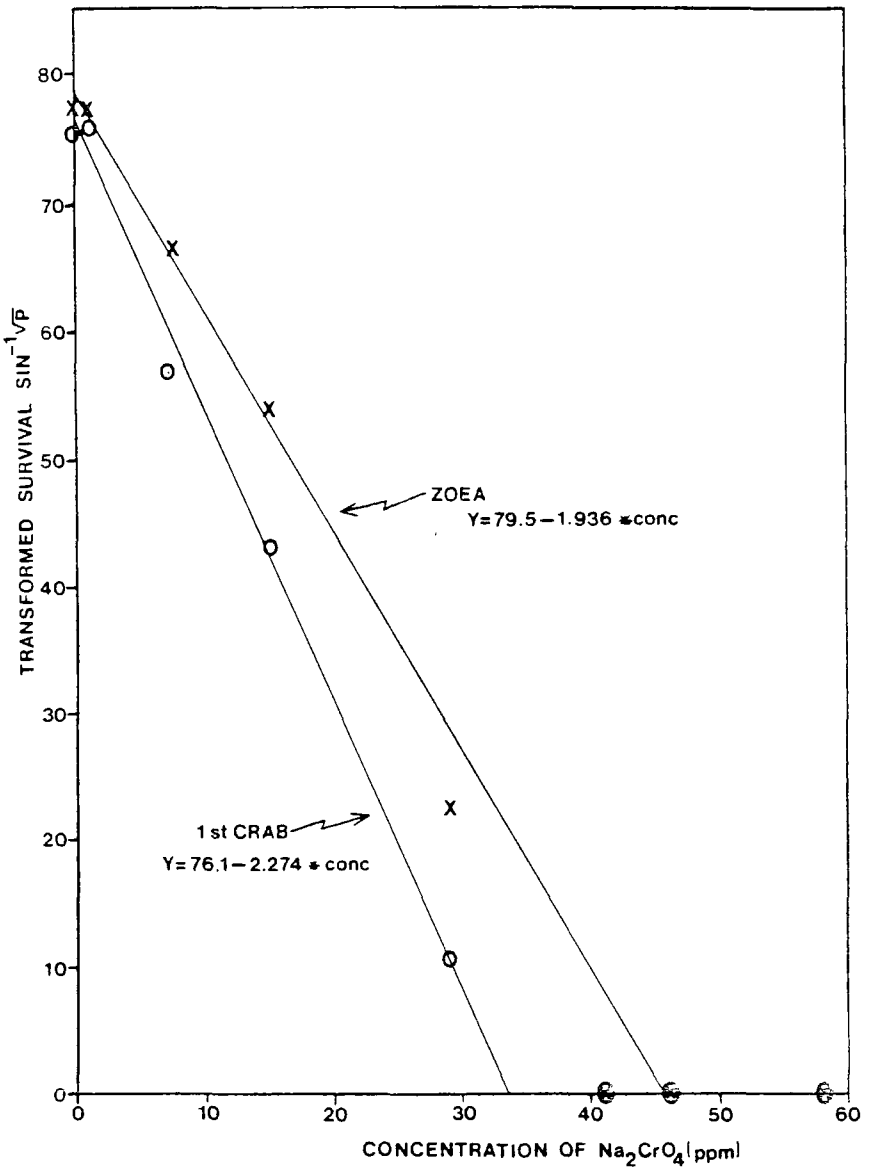

Figure 5. Effect of concentration of hexavalent Na_2CrO_4 in ppm on survival of R. harrisii.
Hatch to megalopa x ——— x
Hatch to 1st crab o ——— o

Duration of R. harrisii Larval Development

Table 11 gives the mean duration in days of R. harrisii zoeal and megalopa development and the mean time in days from hatching to the 1st crab stage for each series reared in seawater control and in different concentrations of Na_2CrO_4. TABLE 12 lists the mean duration of development in days for R. harrisii larvae reared in all series.

Statistical Analysis of R. harrisii Larval Duration

1. Significant linear regressions of both days to megalopa (DZ) and days from hatch to 1st crab (DC) upon Na_2CrO_4 concentrations (CONC) were found with no significant deviations from linearity in concentration of 0 to 29.1 ppm.

$$DZ = 11.90 + 0.120 * CONC$$

$$DC = 18.52 + 0.122 * CONC$$

Where CONC is in ppm of Na_2CrO_4. These results are shown in Figure 6. The regression coefficient may be interpreted as follows:

for DZ : 0.120 ± 0.021 days increase in duration of zoeal development for each ppm added Na_2CrO_4

for DC : 0.122 ± 0.021 days increase in total duration time to 1st crab for each ppm added Na_2CrO_4.

These increases in duration can be scaled up, for example to 10 ppm by multiplication, i.e., DZ 1.20 ± 0.21 days for each 10 ppm Na_2CrO_4 added and DC 1.22 ± 0.21 days increase for each 10 ppm added.

2. Nearly analogous results were obtained when RATE = 100/DAYS was used as the dependent variable:

$$RZ = 8.38 - 0.066 * CONC$$

$$RC = 5.41 - 0.030 * CONC$$

These results are shown in Figure 7.

$$b(RZ) = -0.066 \pm 0.007 \ (DAYS^{-1} * 100)/ppm\ CONC$$

$$b(RC) = -0.030 \pm 0.007 \ (DAYS^{-1} * 100)/ppm\ CONC$$

Mortality of R. harrisii by Larval Stage

Rhithropanopeus harrisii passes through four zoeal stages and a megalopa stage before molting into a 1st crab stage. In an effort to determine if larvae in one or more stages were particularly sensitive to different concentrations of Na_2CrO_4, a record of deaths by stage was made of each of the replicate series of larvae (TABLE 13). Average percent mortality of larvae in

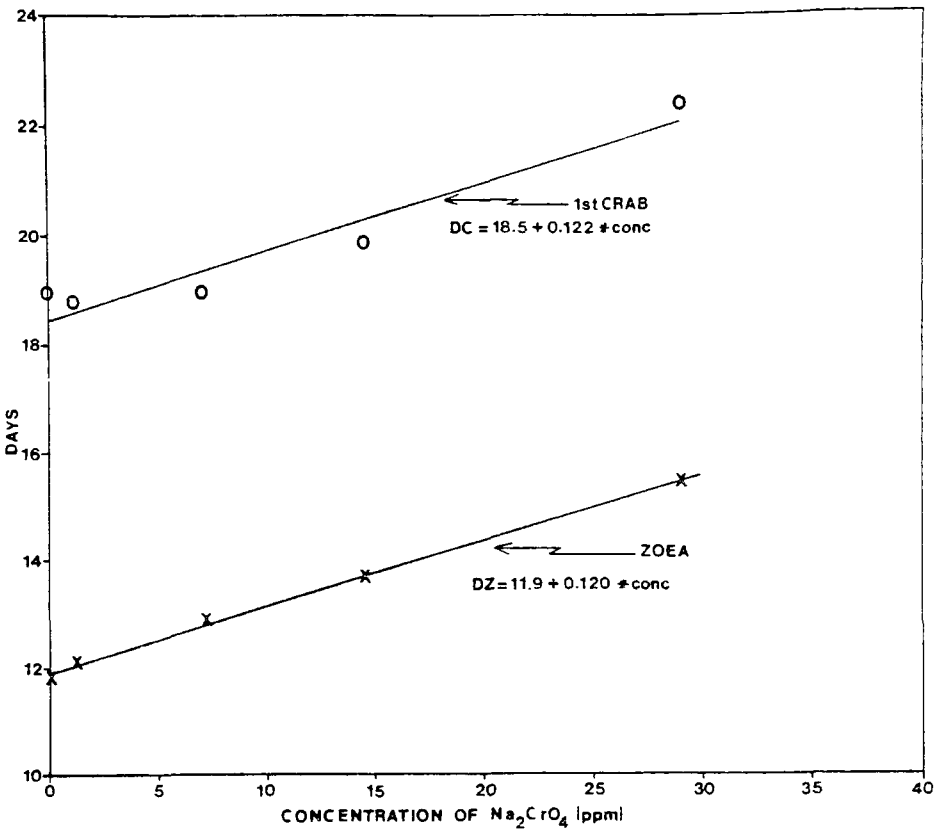

Figure 6. Duration of zoeal development (DZ) and duration to 1st crab (DC) in R. harrisii vs. concentration of Na_2CrO_4 in ppm.
DZ: x———x Hatch to megalopa
DC: o———o Hatch to 1st crab

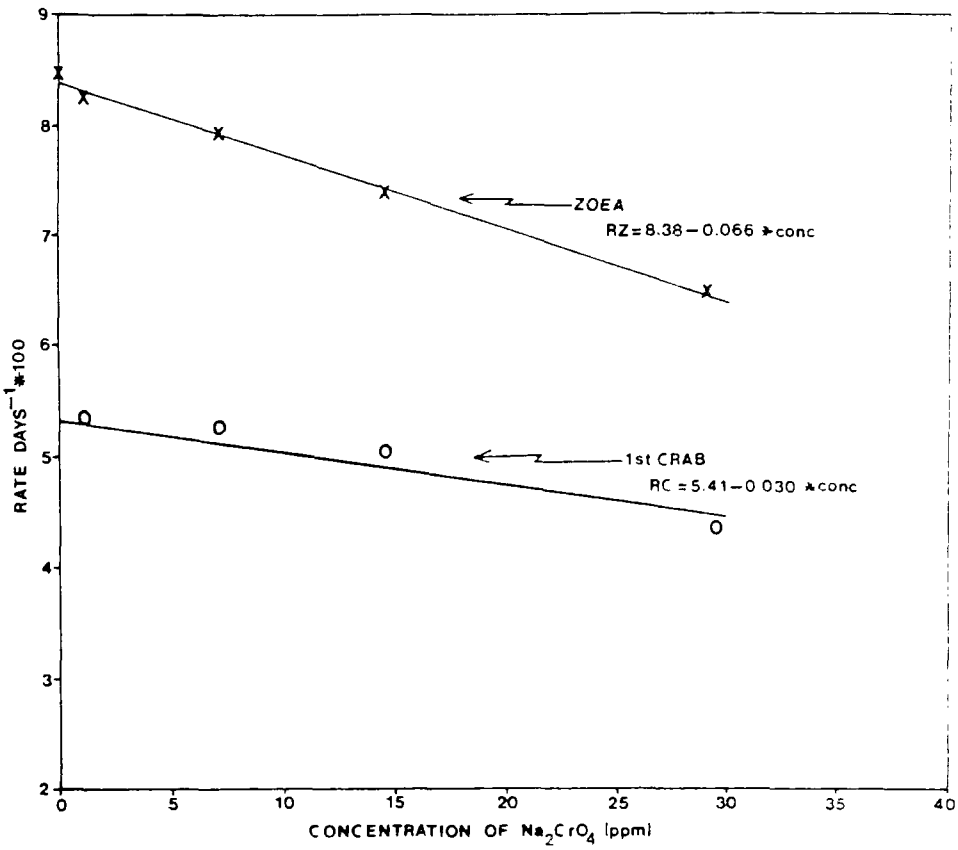

Figure 7. Effect of Na_2CrO_4 in ppm on rate of molting from hatch to megalopa and hatch to 1st crab in R. harrisii.
RZ: x———x Hatch to megalopa
RC: o———o Hatch to 1st crab

TABLE 13. PERCENT MORTALITY IN DEVELOPMENTAL STAGES OF R. harrisii REARED IN SALTWATER CONTROL AND DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM, Na_2CrO_4.

Media	Series	Zoeal Stages I	II	III	IV	Megalopa	Total
Seawater Control	RhI-50	2	2	0	0	0	4
	RhII-50	4	2	2	2	2	12
	RhIII-50	2	2	0	0	4	8
	RhIV-50	2	0	0	4	0	6
	RhV-50	2	0	0	0	2	4
	RhVI-50	2	0	0	2	0	4
1.12 ppm Na_2CrO_4	RhI-50	4	0	0	2	0	6
	RhII-50	6	0	0	4	0	10
	RhIII-50	8	0	0	2	4	14
	RhIV-50	0	2	0	0	2	4
	RhV-50	2	0	0	0	0	2
	RhVI-50	2	0	0	0	0	2
7.17 ppm Na_2CrO_4	RhI-50	8	2	0	0	0	10
	RhII-50	18	0	2	8	34	62
	RhIII-50	10	2	0	4	22	38
	RhIV-50	4	0	2	14	24	44
	RhV-50	0	0	0	6	2	8
	RhVI-50	2	2	4	12	6	26
14.52 ppm Na_2CrO_4	RhI-50	2	0	0	0	16	18
	RhII-50	10	8	6	20	38	82
	RhIII-50	10	0	14	18	10	42
	RhIV-50	2	10	36	34	10	92
	RhV-50	0	2	2	12	8	24
	RhVI-50	2	0	28	8	10	48
29.09 ppm Na_2CrO_4	RhI-50	6	4	10	20	46	86
	RhII-50	24	32	20	20	4	100
	RhIII-50	10	20	70		-	100
	RhIV-50	4	40	44	12	-	100
	RhV-50	2	24	24	16	28	94
	RhVI-50	0	40	6	20	12	78
40.60 ppm Na_2CrO_4	RhIII-50	16	34	44	6	-	100
	RhIV-50	2	88	10	-	-	100
	RhV-50	16	64	18	2	-	100
	RhVI-50	10	86	4	-	-	100

-continued-

TABLE 13 Continued

Media	Series	Zoeal Stages I	II	III	IV	Megalopa	Total
46.40 ppm	RhI-50	18	70	12	-		100
Na_2CrO_4	RhII-50	6	92	2	-	-	100
	RhV-50	44	56	-	-		100
	RhVI-50	14	86	-			100
58.09 ppm	RhI-50	100				-	100
Na_2CrO_4	RhII-50	100	-	-		-	100
	RhV-50	90	10		-		100
	RhVI-50	42	58				100

developmental stages of R. harrisii in all series reared in saltwater control and different concentrations of Na_2CrO_4 is given in TABLE 14. From Figure 8, it can be seen that 1 ppm Na_2CrO_4 is non-toxic, for there is no more mortality in this concentration than in seawater control. There is differential mortality, however, between larvae exposed to concentrations of 1 ppm and larvae exposed to 58 ppm Na_2CrO_4. Concentrations of 7 ppm to 15 ppm Na_2CrO_4 are considered sublethal since more than 10 percent of R. harrisii larvae reached the 1st crab stage. Concentrations of 29, 41, 46, and 58 ppm Na_2CrO_4 are acutely toxic to R. harrisii larvae since less than 10 percent reached the 1st crab stage in 29 ppm and none reached the 1st crab stage in 41, 46 and 58 ppm.

Statistical Analysis of R. harrisii Cumulative Mortality by Stages

The results illustrated in Figure 8 show the effect of Na_2CrO_4 concentrations on the mortality at each stage of development. As indicated earlier (see Statistical Analysis in Materials and Methods) the analyses were performed on the transformed mortality percentages. The means were adjusted to account for the unequal number of replications at some concentrations of Na_2CrO_4 and the tests of significance completed, all in the transformed scale. The means were retransformed to percent mortality for presentation in Figure 8.

Larval Behavior: Swimming Speed of R. harrisii Upon Exposure to Na_2CrO_4

Generally, the swimming speed of seawater control treated R. harrisii larvae (TABLE 15) increases throughout development. Swimming speed is affected by exposure to Na_2CrO_4. However, the direction of the speed change (increase or decrease) and larval sensitivity to sodium chromate changes with developmental stage. For stage I zoeae all test concentrations cause an elevation in swimming speed. This elevation is observed in later stages at

TABLE 14. AVERAGE PERCENT MORTALITY IN DEVELOPMENTAL STAGES OF R. harrisii REARED IN SALTWATER CONTROL AND DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM, Na_2CrO_4.

Media	Series	Zoeal Stages I	II	III	IV	Megalopa	Total
Seawater Control	RhI-50 - RhVI-50	2.3	1.0	0.3	1.3	1.3	6.3
1.12 ppm Na_2CrO_4	RhI-50 - RhVI-50	3.7	0.3	0	1.3	1.0	6.3
7.17 ppm Na_2CrO_4	RhI-50 RhVI-50	7.0	1.0	1.3	7.3	14.7	31.3
14.52 ppm Na_2CrO_4	RhI-50 RhVI-50	4.3	3.3	14.3	15.3	15.3	51.0
29.09 ppm Na_2CrO_4	RhI-50 RhVI-50	7.7	26.7	29.0	14.7	15.0	93.0
40.60 ppm Na_2CrO_4	RhIII-50 RhIV-50 RhV-50 RhVI-50	11.0	68.0	19.0	2.0	-	100.0
46.40 ppm Na_2CrO_4	RhI-50 RhII-50 RhV-50 RhVI-50	20.5	76.0	3.5	-	-	100.0
58.09 ppm Na_2CrO_4	RhI-50 RhII-50 RhV-50 RhVI-50	83.0	17.0	-	-	-	100.0

the two lower concentrations. Depression in swimming rate is first observed in stage II zoeae at the highest test concentration. Stages II and IV zoeae show a depression in swimming rate at the two highest concentrations. Thus the general pattern is for the swimming rate to be elevated by acute exposure to all concentrations and chronic exposure to low sublethal concentrations (1.2 and 7.1 ppm). Swimming rates are only depressed upon chronic exposure to higher sublethal and lethal concentrations (14.5 and 29.1 ppm). These results agree with the normal pattern for the effects of pollutants upon larval swimming. The lower sublethal concentrations cause increases in swimming speed and higher concentrations cause a decline (e.g. Lang et al. 1980).

TABLE 15. SWIMMING SPEED (mm/min) FOR DIFFERENT ZOEAL STAGES OF CONTROL R. harrisii LARVAE AND THOSE TREATED WITH Na_2CrO_4. THE MEAN (M) AND STANDARD DEVIATION (SD) ARE SHOWN. THE SAMPLE SIZE FOR EACH CONDITION IS 60. * INDICATES $P < 0.05$ STATISTICAL DIFFERENCE BETWEEN CONTROL LARVAE AND EXPOSURE TO A PARTICULAR CONCENTRATION WHILE ** IS $P < 0.02$ AND *** IS $P < 0.001$.

Zoeal Stage	Seawater Control M	S.D.	1.12 ppm M	S.D.	7.17 ppm M	S.D.	14.52 ppm M	S.D.	29.09 ppm M	S.D.
I	79.6	36.2	95.0*	36.2	110.4*	43.0	113.8***	46.8	136.4***	52.1
II	112.2	45.6	105.2	40.8	130.4*	51.6	121.8	54.4	85.8***	39.5
III	117.0	47.2	138.8*	65.2	122.4	65.2	98.8	52.6	96.4**	46.0
IV	126.4	56.0	125.6	53.0	152.2*	72.4	104.8*	52.0	104.2*	47.0

Survival of C. sapidus Larvae

The percent survival of C. sapidus larvae from hatching to megalopa and to 1st crab is given for each replicate in TABLE 16. The average percent survival of all series reared in seawater control and four concentrations of Na_2CrO_4 is listed in TABLE 17. There are significant differences between survival of C. sapidus larvae to megalopa and to 1st crab stage reared in seawater control and those larvae reared in Na_2CrO_4 concentrations from 1.1 ppm to 4.7 ppm (TABLE 17). There was better survival of larvae in 1.1 ppm Na_2CrO_4 than in the control and differential survival between 1.1 to 7.2 ppm Na_2CrO_4.

Statistical Analysis of C. sapidus Larval Survival

Statistical analysis indicated that:

(i) survival to megalopa (TRFSZ) and to first crab (TRFSC) are both linearly related to concentration of Na_2CrO_4 in ppm (CONC) in the range 1.1 to 7.2 ppm.

(ii) the slopes of the two lines (Figure 9) differ significantly in the statistical test.

The summary equations are:

Zoea: TRFSZ = 76.8 - 11.1 * CONC

b = -11.1 ± 0.83 Degrees/ppm CONC
or approximately 19% decrease in survival of zoea/ppm increase in Na_2CrO_4 @ 50% survival

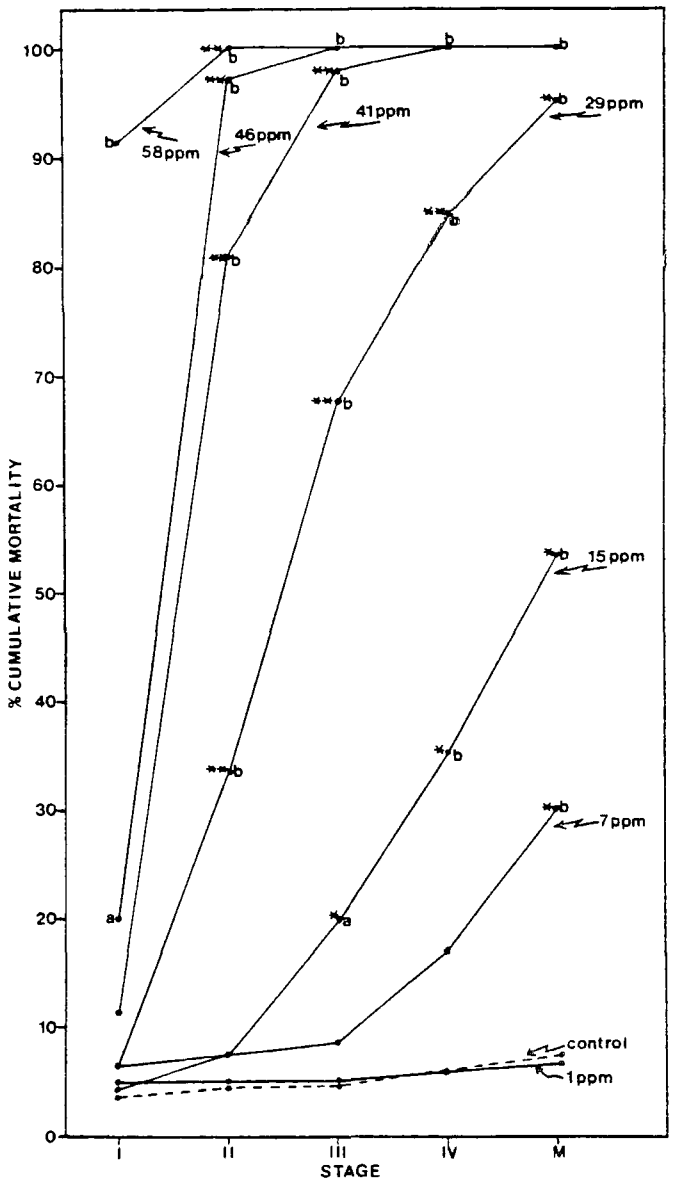

Figure 8. Effect of Na_2CrO_4 in ppm on mortality of R. harrisii.
a. Significantly different from control (0.05)
b. Significantly different from control (0.01)
*. Significant increase over previous stage (0.05)
**. Significant increase over previous stage (0.01)

TABLE 16. PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF THREE SERIES (Cs I-III) OF Callinectes sapidus REARED IN SEAWATER CONTROL AND IN DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM, Na_2CrO_4

Culture Media Salinity 30°/°° Temp. 25°C	Initial No. of larvae per series	% Survival to		Mean Duration of Development in days		
		Megalopa	1st crab	Zoea	Megalopa	Hatch to 1st Crab
Seawater Control	CsI-50	64	36	34.8	7.6	40.5
	CsII-50	58	36	32.2	7.7	40.0
	CsIII-50	62	42	33.5	7.4	38.3
1.1 ppm	CsI-50	88	54	33.6	7.0	41.5
Na_2CrO_4	CsII-50	78	46	31.9	7.6	39.6
	CsIII-50	76	34	37.9	6.9	44.8
2.4 ppm	CsI-50	74	64	36.0	7.3	43.1
Na_2CrO_4	CsII-50	72	32	34.1	8.1	41.6
	CsIII-50	50	28	38.2	7.4	44.9
4.7 ppm	CsI-50	24	16	41.8	8.3	47.3
Na_2CrO_4	CsII-50	18	6	39.0	7.7	43.3
	CsIII-50	-	-			-
7.2 ppm	CsI-50	-	-	-		-
Na_2CrO_4	CsII-50	-	-	-		
	CsIII-50	-		-		

First Crab: TRFSC = 52.5 - 7.5 * CONC

b = -7.5 ± 0.83 Degrees/ppm CONC
or approximately 13% decrease in survival to first crab/ppm increase in Na_2CrO_4 @ 50% survival.

Estimated LC50 values were obtained by setting each equation equal to 45 degrees (50% survival) and solving each equation for the value of CONC.

Estimated LC50 values were:

Zoea: 2.9 ppm Na_2CrO_4

First Crab: 1.0 ppm Na_2CrO_4

Duration of C. sapidus Larval Development

TABLE 16 gives the mean duration in days of zoeal and megalopa development for C. sapidus and the mean time in days from hatching to the 1st crab stage for each series reared in seawater control and in different

TABLE 17. AVERAGE PERCENT SURVIVAL AND AVERAGE DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF THREE SERIES (Cs I-III) OF Callinectes sapidus REARED IN SEAWATER CONTROL AND IN DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM, Na_2CrO_4

Culture Media Salinity 30°/°° Temp. 25°C	Initial No. of larvae per series	% Survival to		Mean Duration of Development in days		
		Megalopa	1st crab	Zoea	Megalopa	Hatch to 1st Crab
Seawater Control	CsI-50 CsII-50 CsIII-50	61.3	38.0	33.5	7.6	39.6
1.1 ppm Na_2CrO_4	CsI-50 CsII-50 CsIII-50	80.6	44.7	34.5	7.2	42.0
2.4 ppm Na_2CrO_4	CsI-50 CsII-50 CsIII-50	65.3	41.3	36.1	7.6	43.2
4.7 ppm Na_2CrO_4	CsI-50 CsII-50 CsIII-50	14.0	7.3	40.0	8.0	45.3
7.2 ppm Na_2CrO_4	CsI-50 CsII-50 CsIII-50	-	-	-		-

concentrations of Na_2CrO_4. TABLE 17 lists the mean duration of development in days for C. sapidus larvae reared in all series.

Statistical Analysis of C. sapidus Larval Duration

1. Significant linear regressions of Days to Megalopa (DZ) and Days to First Crab (DC) upon concentration of Na_2CrO_4 in ppm (CONC) were found. No significant deviations from linearity occurred in the range 0 to 4.7 ppm.

   The most compact summary is in these equations:

$$DZ = 32.9 + 1.65 * CONC$$

$$DC = 40.0 + 1.31 * CONC$$

   Where CONC is in ppm of Na_2CrO_4. These results are shown in Figure 10. The regression coefficient may be interpreted as follows:

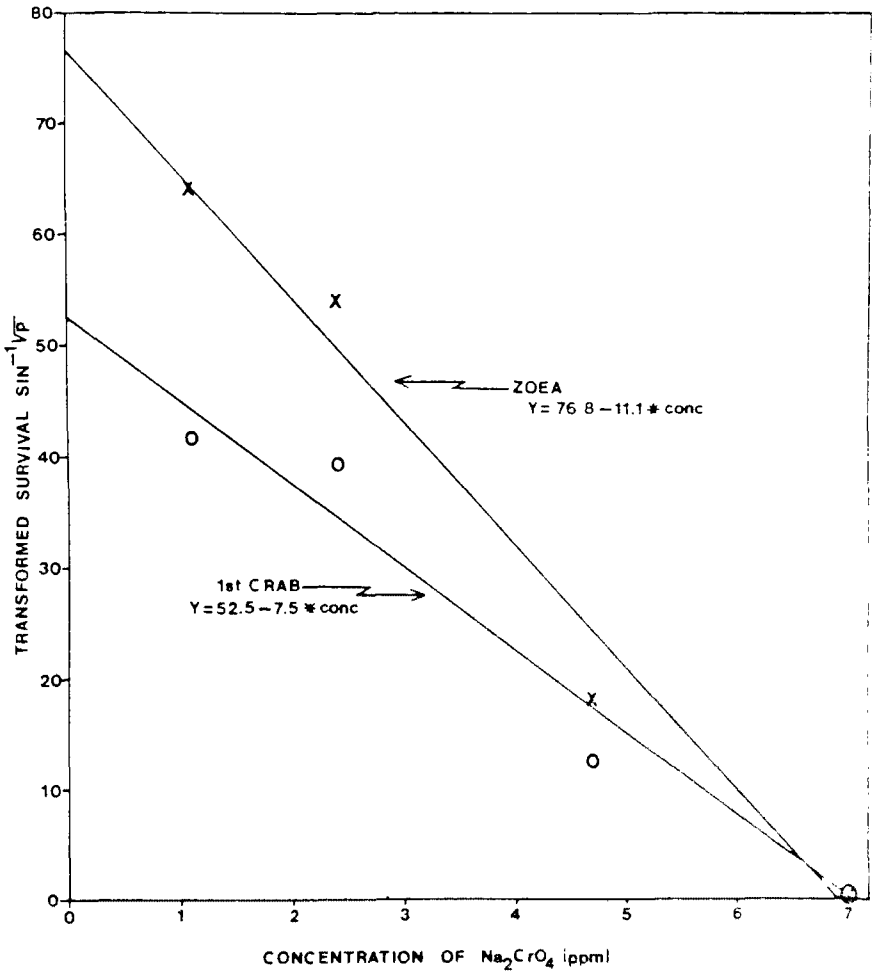

Figure 9. Effect of concentration of Na_2CrO_4 in ppm on survival of C. sapidus.
R. harrisii.
Hatch to megalopa x————x
Hatch to 1st crab o————o

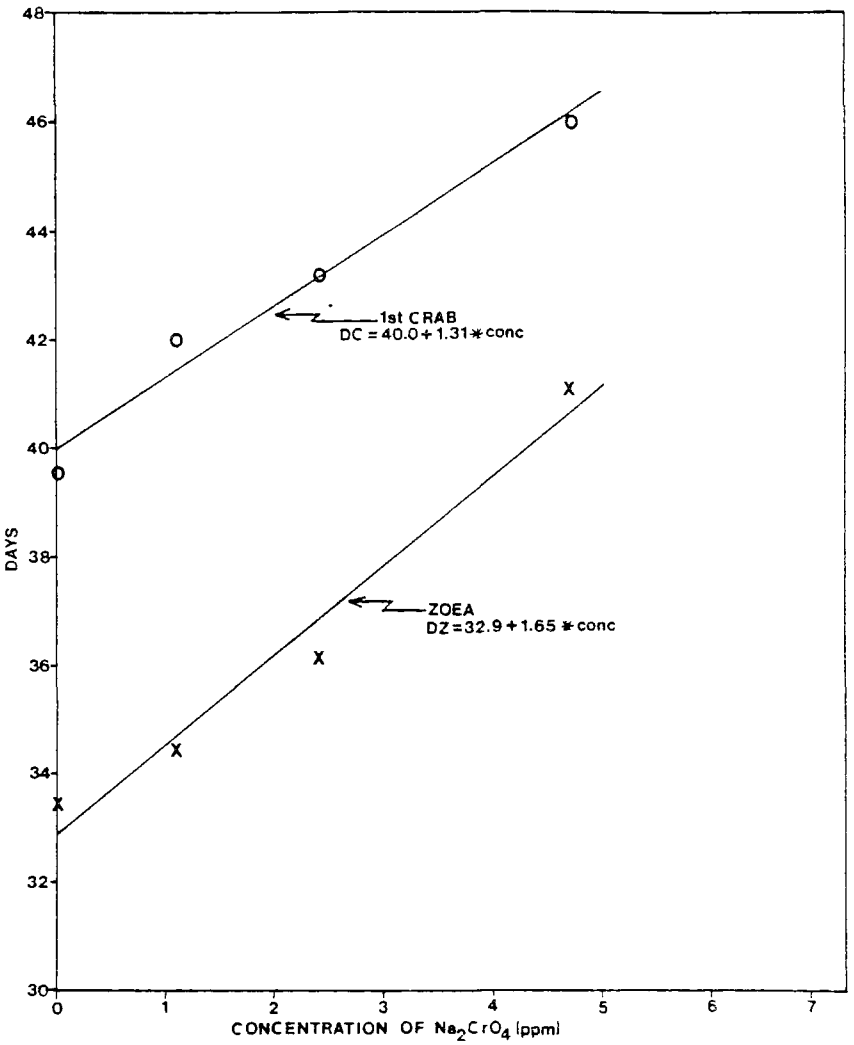

Figure 10. Duration of zoeal development (DZ) and duration to 1st crab (DC) in C. sapidus vs. concentration of Na_2CrO_4 in ppm.
DZ: x———x Hatch to megalopa
DC: o———o Hatch to 1st crab

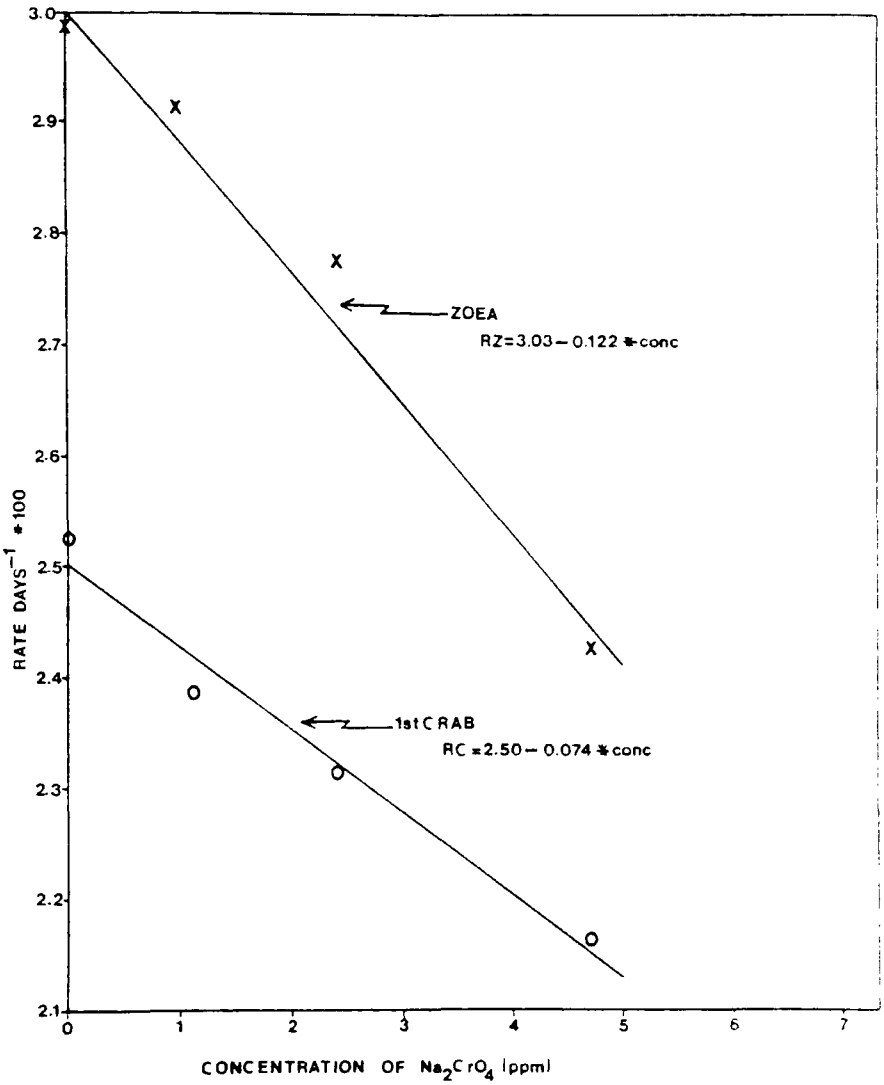

Figure 11. Effect of Na_2CrO_4 in ppm on rate of molting from hatch to megalopa and hatch to 1st crab in C. sapidus.
RZ: x———x Hatch to megalopa
RC: o———o Hatch to 1st crab

TABLE 18. PERCENT MORTALITY IN DEVELOPMENTAL STAGES OF THREE SERIES (Cs I-III) OF C. sapidus REARED IN SALTWATER CONTROL AND DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM, Na_2CrO_4

Media	Series	Zoeal Stages I	II	III	IV	V	VI	VII	VIII	Megalopa	Total
Seawater Control	CsI-50	2	16	4	4	0	4	6	0	28	64
	CsII-50	8	10	14	6	0	2	2	0	22	64
	CsIII-50	4	6	20	2	6	0	0	0	20	58
1.1 ppm Na_2CrO_4	CsI-50	4	0	0	4	0	4	0	0	34	46
	CsII-50	6	6	6	0	0	0	4	0	32	54
	CsIII-50	4	2	2	2	4	4	4	2	42	66
2.4 ppm Na_2CrO_4	CsI-50	4	8	4	4	4	2	0	0	10	36
	CsII-50	6	2	14	2	2	0	2	0	40	68
	CsIII-50	14	6	18	2	0	4	4	2	22	72
4.7 ppm Na_2CrO_4	CsI-50	18	4	12	24	12	6	0	0	8	84
	CsII-50	10	8	16	16	12	12	6	2	12	94
	CsIII-50	2	6	30	38	20	4	0	0	0	100
7.2 ppm Na_2CrO_4	CsI-50	14	6	54	22	4	0	0	0	0	100
	CsII-50	12	18	60	8	2	0	0	0	0	100
	CsIII-50	0	2	98	0	0	0	0	0	0	100

for DZ: 1.65 ± 0.29 days increase in duration of zoeal development for each ppm added Na_2CrO_4

for DC: 1.31 ± 0.29 days increase in total duration time to 1st crab for each ppm added Na_2CrO_4.

2. Nearly analagous results were obtained when RATE = 100/DAYS was used as the dependent variable:

$$RZ = 3.03 - 0.122 * CONC$$

$$RC = 2.50 - 0.074 * CONC$$

These results are shown in Figure 11.

$$b(RZ) = -0.122 \pm 0.020 \ (DAYS^{-1} * 100)/ppm\ CONC$$

$$b(RC) = -0.074 \pm 0.020 \ (DAYS^{-1} * 100)/ppm\ CONC$$

TABLE 19. AVERAGE PERCENT MORTALITY IN DEVELOPMENTAL STAGES OF THREE SERIES (CsI-III) OF C. sapidus REARED IN SALTWATER CONTROL AND DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM, Na_2CrO_4

Media	Series	Zoeal Stages I	II	III	IV	V	VI	VII	VIII	Megalopa	Total
Seawater Control	CsI-50 CsII-50 CsIII-50	4.7	10.7	12.7	4.0	2.0	2.0	2.7	0	27.3	62.0
1.1 ppm Na_2CrO_4	CsI-50 CsII-50 CsIII-50	4.7	2.7	2.7	1.3	2.0	2.7	2.7	0.7	36.0	55.3
2.4 ppm Na_2CrO_4	CsI-50 CsII-50 CsIII-50	8.0	5.3	12.0	2.7	2.0	2.0	2.0	0.7	24.0	58.7
4.7 ppm Na_2CrO_4	CsI-50 CsII-50 CsIII-50	10.0	6.0	19.3	26.0	14.7	7.3	2.0	0.7	6.7	92.7
7.2 ppm Na_2CrO_4	CsI-50 CsII-50 CsIII-50	8.6	8.7	70.7	10.0	2.0	0	0	0	0	100

Mortality of C. sapidus by Larval Stage

Callinectes sapidus passes through seven to eight zoeal stages and a megalopa stage before molting into a 1st crab stage. In an effort to determine if larvae in one or more stages were particularly sensitive to different concentrations of Na_2CrO_4, deaths were recorded by stage for each replicate series of larvae (TABLE 18). Average percent mortality of larvae in developmental stages of C. sapidus in all series reared in saltwater control and different concentrations of Na_2CrO_4 is given in TABLE 19. From Figure 12, it can be seen that there is significantly less mortality in 1.1 ppm Na_2CrO_4 than in seawater control. There is also less mortality in 2.4 ppm Na_2CrO_4, but it is not significantly different from the control and hence it is considered nontoxic. There is differential mortality from concentrations of 4.7 to 7.2 ppm Na_2CrO_4, and these concentrations are considered acutely toxic since less than 10 percent of C. sapidus larvae reached the 1st crab stage.

Statistical Analysis of C. sapidus Cumulative Mortality by Stages

The results illustrated in Figure 12 show the effect of Na_2CrO_4 concentrations on the mortality at each stage of C. sapidus larval development. Zoeae in zoeal stage III were extremely sensitive to 7.2 ppm

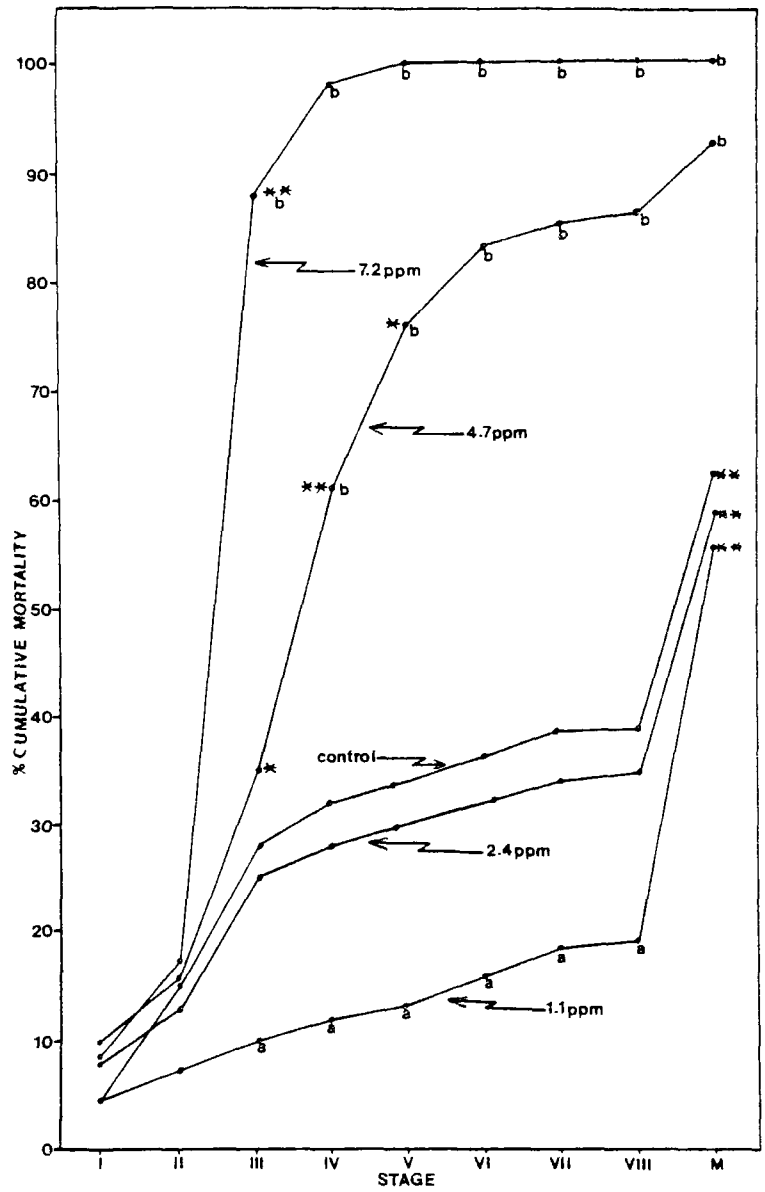

Figure 12. Effect of Na_2CrO_4 in ppm on mortality of C. sapidus.

a. Significantly different from control (0.05)
b. Significantly different from control (0.01)
*. Significant increase over previous stage (0.05)
**. Significant increase over previous stage (0.01)

Na_2CrO_4 (TABLE 19 and Figure 12). In 4.7 ppm, zoeae in zoeal stages III, IV and V were the most sensitive and mortality was significantly different from the control in all developmental stages after zoeal stage III (Figure 12). In the control, 1.1 and 2.4 ppm Na_2CrO_4 there was a very significant increase in mortality in the megalopa stage compared to the previous stage (TABLE 19 and Figure 12).

DISCUSSION

Survival

The range of concentrations of Na_2CrO_4 in which development of Rhithropanopeus harrisii occurred from hatch to 1st crab was from 1.1 to 29.1 ppm Na_2CrO_4 (TABLE 12), whereas in Callinectes sapidus it was from 1.1 to 4.7 ppm (TABLE 17). Na_2CrO_4 concentrations of 1.1 ppm to R. harrisii and 1.1 and 2.4 ppm to C. sapidus were nontoxic. Actually survival of C. sapidus larvae was significantly better in 1.1 ppm Na_2CrO_4 and somewhat better in 2.4 ppm than in seawater control, possibly because these concentrations may have killed bacteria or neutralized other toxic substances which were in seawater. Concentrations of 7.2 and 14.5 ppm Na_2CrO_4 were sublethal concentrations to R. harrisii, because more than 10% reached the 1st crab stage and there was a reduction in survival with an increase in concentration compared to survival in seawater control (Epifanio, 1971; Bookhout and Costlow, 1975). Sublethal concentrations for C. sapidus would probably be between 2.4 and 4.7 ppm Na_2CrO_4, but they were not employed in this investigation. Acutely toxic concentrations of Na_2CrO_4 to R. harrisii were 29.1, 40.6, 46.4 and 58.1 ppm, for only 7% of the larvae reached the 1st crab stage in 29.1 ppm and no larvae reached the 1st crab stage in the other concentrations of Na_2CrO_4. The acutely toxic concentrations to C. sapidus were 4.7 ppm, in which 7.3% of the larvae became 1st crabs, and 7.2 ppm Na_2CrO_4, in which no larvae reached the 1st crab stage.

The estimated 96-h LC50 for zoeal development from hatch to megalopa was 17.8 ppm Na_2CrO_4 for R. harrisii and 2.9 ppm for C. sapidus, and the estimated 96-h LC50 for development from hatch to 1st crab was 13.7 ppm Na_2CrO_4 for R. harrisii and 1.0 ppm for C. sapidus.

Comparative Toxicity

Most of the research on the effect of hexavalent chromium has been acute toxicity studies on adult organisms, not on larvae. Eisler and Hennekey (1977) using K_2CrO_4 reported the 7-day LC-100 for the sandworm, Nereis virens, was 5 ppm; 20 ppm for the hermit crab, Pagurus longicarpus; 50 ppm for the soft shell clam, Mya arenaria; 20 ppm for the starfish, Asterias forbesi; 20 ppm for the snail, Nassarius obsoletus; and 100 ppm for the mummichog Fundulus heteroclitus. All of these species with the exception of Nereis virens were less susceptible to hexavalent chromium than C. sapidus during larval development, but only the soft shell clam and the mummichog were less susceptible to hexavalent chromium than R. harrisii during larval development.

In making comparisons of the effects of Cr^{+6} on other crustaceans, differences in toxicity may depend upon the temperature and salinity of the medium. Fales (1978) found that the susceptibility of the grass shrimp, Palaemonetes pugio, to potassium chromate was greatest at 25°C and a 10°/$_{oo}$ salinity and least at 10°C and 20°/$_{oo}$. In the first case the 48-h TL_m (median tolerance limit) value was 21 ± 4 mg Cr/l and in the second case the TL_m value was 147 ± 16 mg Cr/l. Frank and Robertson (1979) also reported the influence of salinity on the toxicity of Cr^{+6} to juvenile blue crabs, Callinectes sapidus. Using $K_2Cr_2O_7$, they found that the 96-h LC50 to juvenile crabs was 34.2 ppm Cr^{+6} in a salinity of 1°/$_{oo}$, whereas the 96-h LC50 to juvenile blue crabs of the same size was 98 ppm Cr^{+6} in 35°/$_{oo}$ salinity.

From the above discussion, the implications are that when R. harrisii and C. sapidus are reared from hatching to 1st crab at a temperature of 25°C, Cr^{+6} would tend to adversely affect development. Since R. harrisii and C. sapidus were reared in salinities of 20°/$_{oo}$ and 30°/$_{oo}$, respectively, however, these high salinities might counter the adverse effects of high temperatures.

Sublethal Effects

The increase in duration with each increase in Na_2CrO_4 in zoeal development from hatching to megalopa and in development from hatching to 1st crab stage in R. harrisii (TABLE 12 and Figure 6) and C. sapidus (TABLE 17 and Figure 10) is considered a sublethal effect of hexavalent chromium. Swimming behavior of R. harrisii is also modified by Na_2CrO_4. Exposure to sublethal concentrations of Na_2CrO_4 caused an elevation in swimming speed, while near lethal concentrations produced a depression (TABLE 15).

Environmental Implications

Sodium chromate, Na_2CrO_4, is one of the potentially hazardous materials being discharged into saline environments from metal processing facilities, chemical industries and other sources. The total chromium in sodium chromate is 32 percent by weight according to Dr. Tacy of Hazleton Laboratories. In chronic bioassays on the effects of Na_2CrO_4 on the complete larval development of R. harrisii and C. sapidus, it was observed that 1.1 ppm Na_2CrO_4 with total chromium of 0.36 ppm was nontoxic during the complete larval development of R. harrisii and C. sapidus. This concentration was also nontoxic to the fathead minnow, Pimephales promeles, in the first and second generation in hard water (Pickering, 1980). Concentrations of 7.2 ppm Na_2CrO_4 with total chromium of 2.3 ppm and 14.5 ppm Na_2CrO_4 with total chromium of 4.66 ppm were sublethal to R. harrisii, and it was estimated that concentrations between 2.4 ppm Na_2CrO_4 with total chromium of 0.77 ppm and 4.7 ppm Na_2CrO_4 with total chromium of 1.5 ppm would be sublethal to C. sapidus. These concentrations would undoubtedly be absorbed by crab larvae, and they would bioaccumulate in the tissues of the larvae in the course of time. Eventually the bioaccumulated chromium would produce stress and more mortality than in the control, especially in later zoeal stages as shown in Figure 8. Acutely toxic concentrations in which less than 10% reach the 1st crab stage, ranged from 29.1 ppm Na_2CrO_4 with total chromium of 9.31 ppm to 58.1 ppm Na_2CrO_4 with total chromium of 18.65 ppm for R. harrisii and 4.7 ppm Na_2CrO_4 with total chromium of 1.5 ppm and 7.2 ppm Na_2CrO_4 with 2.3 ppm total chromium for

C. sapidus. In acutely toxic concentrations, there is marked mortality in the first few zoeal stages as shown in Figures 8 and 12. The larval development of R. harrisii might be considered one of the most resistant to Na_2CrO_4 and the larval development of C. sapidus among the most sensitive.

There is a question in the literature whether hexavalent chromium, Na_2CrO_4, which is incorporated into chrome lignosulfonate or ferrochrome lignosulfonate before they are added to drilling fluids, has any detrimental effect on the complete development of crabs or other planktonic organisms. If hexavalent chromium plays an active part, it is more indirect and complex than when Na_2CrO_4 is discharged from manufacturing plants into the marine environment.

It is generally assumed that most of the chromium in the discharge of drilling fluids is trivalent chromium (Cr^{+3}) which is not as readily bioavailable or toxic to planktonic organisms compared to hexavalent chromium (Cr^{+6}), which will pass through biological membranes readily (Mertz, 1969) and is known to be toxic to marine organisms. If discharges of whole used lignosulfonate or chrome lignosulfonate type muds were emitted at the same time as additions of chrome lignosulfonate or ferrochrome lignosulfonate were being made, it is possible that both Cr^{+3} and Cr^{+6} would be present in the discharge. Initially both chrome lignosulfonate and ferrochrome lignosulfonate, "Q-Broxin," contain hexavalent chromate salts, but at temperatures between 120 to 175°C hexavalent chromium is converted to the trivalent state. The property of these two additives can be restored at temperatures between 120 to 175°C by adding more hexavalent salts (Liss et al., 1980).

Neff et al. (1981) found approximately 500 ppm total chromium in whole used chromium lignosulfonate drilling fluid and less than 1 ppm total chromium in the filtered mud aqueous fraction (MAF) which is found in the upper turbidity plume together with the suspended particulate phase (SPP). It is these two mud fractions which were found to be toxic to R. harrisii and C. sapidus as described in Section 4 of this manuscript. Carr et al. (1981) evaluated the bioavailability of chromium from chrome lignosulfonate drilling fluid to five species of marine invertebrates. The shrimp, Palaemonetes pugio, exposed to MAF with a concentration of 0.25 ppm chromium for seven days, accumulated 23.7 ppm, but released it within 96 hours of depuration to control levels. Clams, Rangia cuneata, exposed to MAF for 16 days, accumulated up to 19 ppm chromium in their tissues. When the clams were returned to clean water, they rapidly lost about half of the chromium, but retained 11 ppm even after 11 days. McCulloch et al.(1980) confirmed the findings of Carr et al. (1981) in respect to the bioavailability of chromium when Rangia was exposed to MAF. They also found that Rangia could accumulate chromium from different concentrations of MAF and retain about half after depuration. They also exposed oyster spat, Crassostrea gigas, to MAF and SPP, and found that they accumulated more chromium from SPP than MAF. Thus suggesting that they had a limited ability to accumulate particle-adsorbed chromium, possibly by pinocytosis, whereas Rangia might absorb moderate concentrations of chromium, possibly chiefly in the form of soluble chrome lignosulfonate complex (Knox, 1978). Neff et al. (1979), Carr et al. (1981)

and McCulloch et al. (1980) reported that a preliminary analysis of total chromium in MAF revealed that more than 75% of it was in the trivalent state.

It seems reasonable to assume that if trivalent chromium in MAF can be accumulated up to 19 ppm in the tissues of Rangia (Carr et al., 1981), and if Na_2Cr_4 were also present in the medium, even at a lower concentration than trivalent chromium, it would be absorbed more readily and the total chromium bioaccumulated would be greater. This condition could account for the variations in bioaccumulation of chromium reported in the literature. Although Palaemonetes accumulated 23.7 ppm chromium in seven days, it was released within a 96-h depuration period to control levels. This may imply that Palaemonetes merely adsorbed trivalent chromium and never absorbed it into tissue, possibly because its cells could not absorb trivalent chromium, nor were the cells able to take in chromium associated particles of clay by pinocytosis. If this conclusion is valid, it is very possible that crab larvae and Palaemonetes could only absorb hexavalent chromium.

It has recently been suggested that after drilling fluids are discharged into the ocean, chromium and associated material are released slowly in soluble form from clay particles into the water (Knox, 1978). Once freed from clay particles, Cr^{+3} through slow oxidation may revert to Cr^{+6} as Fukai and Vas (1969), Schroeder and Lee (1975), and Cranston and Murray (1980) reported. This would take place over 7 to 30 days and involve from 3 to 7% of trivalent chromium.

The decrease in concentration of suspended solids in the upper turbidity plume from the point of discharge peripherally to background levels has been discussed in Section 4 of this report. For most discharges the background concentration for chromium has been reported to be approximately 100 to 150 meters from the point of discharge. The distance will vary depending on the amount and rate of discharge, as well as the currents (Ray and Meek, 1980). Within this area entrained crab larvae might absorb and bioaccumulate Cr^{+6} as Cr^{+3}, as occurs when Cr^{+6} in human blood plasma enters the corpuscles and is quickly converted to Cr^{+3} (NAS, 1974). It is questionable whether crab larvae would remain in the upper turbidity plume long enough to bioaccumulate enough chromium to kill the larvae or to produce sublethal stress. This is especially true since the initial response of the larvae upon exposure to Cr^{+6} concentrations is an increase in random swimming speed (TABLE 15) which increases the probability of the larvae leaving the area. Hence, it is probable that chromium in drilling fluids, whether Cr^{+3} or Cr^{+6}, is not likely to reduce the population of crab larvae and other planktonic organisms in the area around oil wells except possibly in the immediate vicinity of the discharge pipes.

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GLOSSARY

acute toxicity tests: short-term exposure to concentrations of toxicant which will be lethal to 50% of the larvae in a short interval of time; 24 h, 48 h, or 96 h.

acutely toxic concentrations: concentrations of pollutant in which less than 10% of the larvae survive to the 1st crab stage.

analysis of variance: a special application of the linear models technique which can be used effectively when the experimental design is balanced with respect to factors and replication.

chronic tests: long-term exposure to toxicant.

cummulative mortality: the total number of larval deaths incurred at any given stage of development expressed as a percent of the initial number of larvae.

differential survival: reduction of survival with each increase in pollutant.

dosage-response relationship: the characterization of the change in response (e.g. survival) with changing stimulus (e.g. concentrations of pollutant). Typically such responses vary from 0% at some threshold level of the stimulant to 100% at some uniformly lethal level of the stimulant. An intermediate point is the ED50, the "effective dose" at which 50% of the organisms react to the stimulant.

first crab stage: first stage after molt from megalopa; has adult morphology with abdomen bent under cephalothorax, but is sexually immature.

fitting a linear regression: another special application of the linear models technique where the response variable is a simple linear function of a single independent variable, $y = \alpha + \beta x + \varepsilon$, and the relevant statistics are estimates of the parameters, α, β, and σ^2, the variance of the random error, ε.

general linear models technique: an attempt to characterize a given response (e.g. survival) as a linear function of factors, experimentally imposed and environmentally existent, and their interactions. Statistical analysis of the resulting model quantitatively evaluates the relative importance of the several factors and the experimental errors.

h: hour

LC50: lethal concentration; the concentration of toxicant in water estimated to be lethal to 50 percent of test aimals for a specified period of exposure.

megalopa: stage of development of a crab between last zoeal stage and 1st crab stage; is dorso-ventrally depressed; has all cephalothoracic and abdominal appendages present and functional; and has extended abdomen.

g/g: micrograms per gram = parts per million.

g/l: micrograms per liter = parts per billion.

mg/l: milligrams per liter = parts per million.

molt: the process of shedding the exoskeleton which is necessary for growth during larval and juvenile development in arthropods, including crustaceans.

mud aqueous fraction (MAF): one part by volume of used drilling mud with nine parts seawater of the appropriate salinity. The mixture is stirred thoroughly with an electric mixer and then allowed to settle for 20 hours. The dark colored aqueous layer is siphoned off for immediate use in bioassays. The undiluted supernate is 100% MAF and contains the water soluble and fine particulate fractions of 100,000 ppm mud in water (Neff et al. 1980). Other fractions are prepared by diluting 100% MAF with seawater.

ppb: parts per billion.

ppm: parts per million.

°/₀₀: parts per thousand.

regression coefficient, in the linear regression model: the regression coefficient of the independent variable and the slope of the straight line relating y to x. If y is measured in 'DAYS' and x in 'ppm,' the units of slope are DAYS/ppm.

sublethal concentrations: concentrations of pollutant in which 10% or more of the larvae survive to the 1st crab stage.

sublethal effects: effects in larvae reared in sublethal concentrations, but not in acetone control; they become more pronounced as concentrations are increased.

sub-plot error: the component of experimental error that affects the repeated measurements on the same experimental unit, e.g. cumulative mortality of an original unit of 100 larvae.

suspended particulate phase (SPP): one part of volume of used drilling mud with nine parts seawater of appropriate salinity. The mud-seawater slurry is air mixed with filtered compressed air for 30 minutes, with manual stirring every 10 minutes. After aeration the suspension is allowed to settle before the supernate (100% SPP) is siphoned off for immediate use in bioassays. The SPP resembles the MAF except that SPP contains a higher concentration of particulates and a lower concentration of volatiles.

technique of split-plot analysis of variance: sometimes called a "repeated measurement design" when successive measurements are taken on the same experimental unit, e.g., survival at each stage of development. The resulting analysis provides for two or more levels at which different components of experimental error may affect the response.

transformed to angular scale: the transformation of data expressed in 'percent' to a new scale where the $\sqrt{\text{percent}}$ is treated as the sine of an angle. While 'percent' varies from 0 to 100 the corresponding 'angles' vary from 0° to 90°. The angular scale is more amenable to statistical analysis because the sampling variance is approximately constant whereas the variance in the percent scale is not.

weighted standard error: a standard error that combines the estimate of error associated with experimental units treated alike (whole-plot error) with the estimate of sub-plot error to provide an appropriate basis for comparing sub-plot means at different levels of whole-plot factors, e.g., to compare the mortality at a given zoeal stage at several different concentrations of pollutant.

zoea(e): a planktotropic larval stage of a crab with a laterally compressed cephalothorax and abdomen, and two thoracic appendages (maxillipeds) for swimming.

zoeal development: refers to all zoeal stages from time of hatching to megalopa stage (i.e., four zoeal stages in R. harrisii and seven to eight zoeal stages in C. sapidus).

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C.G. Bookhout, Robert Monroe*, Richard Foward, J.D. Costlow, Jr., *North Carolina State Univ.

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16. ABSTRACT

The mud aqueous fractions (MAF) and suspended particulate phase (SPP) of lignosulfonate type mud were nontoxic to the complete larval development of Rhithropanopeus harrisii. Five percent MAF and SPP were not toxic to Callinectes sapidus. Differential survival of C. sapidus larvae occurred from 5 to 50% MAF and SPP. No larvae reached the 1st crab stage in 100% MAF and SPP. Statistical analyses of the data on survival, mortality and behavior are presented.

Survival of R. harrisii from hatching to 1st crab stage occurred in 1.1 to 29.1 ppm Na_2CrO_4. Estimated LC_{50} for complete zoeal development was 17.8 ppm Na_2CrO_4 and was 13.7 for development to 1st crab stage. A concentration of 1.1 ppm was nontoxic, 7.2 and 14.5 Na_2CrO_4 were sublethal and concentrations of 29.1 to 58.1 ppm were acutely toxic. Low concentrations of Na_2CrO_4 caused an increase in swimming speed and high concentrations caused a decline.

Survival of Callinectes sapidus occurred in 1.1 to 4.7 Na_2CrO_4. The LC_{50} for complete zoeal development was estimated to be 2.9 ppm and the LC_{50} for development to 1st crab stage was estimated to be 1.0 ppm. Statistical analyses of the data on survival, duration and mortality of larvae are presented.

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6 Author, Organization, and Address

C.G. Bookhout, Robert Monroe, Richard Foward, and J.D. Costlow, Jr.
Duke University Marine Laboratory
Beaufort, North Carolina 28516

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