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Project Summary

Effects of Soluble Fractions of Drilling Fluids and Hexavalent Chromium on the Development of the Crabs, *Rhithropanopeus harrisii* and *Callinectes sapidus*

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This study is part of a series of investigations undertaken to determine the effect of the discharge of drilling fluids on marine fauna. The first division of this project was to determine the range of concentrations of the mud aqueous fraction (MAF) and the suspended particulate phase (SPP) of a low-density 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. As far as is known, this is the first investigation on the effect of MAF and SPP, soluble fractions of whole mud, on the complete larval development of crabs. These fractions would be found in the upper plume of discharges of an oil well.

The percent survival from hatching to megalopa and to 1st crab stage was 90% or over in seawater control and in concentrations from 5% (5,000 ppm) to 100% (100,000 ppm) MAF and SPP in three replicate series of *R. harrisii* larvae tested.

There was differential survival of *Callinectes sapidus* from hatching to 1st crab stage in concentrations from 5% to 50% MAF and SPP, but no larvae reached the 1st crab stage in 100% MAF or SPP. Statistical analyses of the data on survival, mortality and behavior are presented.

A second division of the project was to determine the effect of hexavalent chromium, Na_2CrO_4, on the complete larval development of *R. harrisii* and *C. sapidus* from the time of hatching to 1st crab stage. The effects of different concentrations of Na_2CrO_4 on survival and duration from hatch to megalopa and hatch to 1st crab stage are given, and the most sensitive stages of larval development were determined for each species. Statistical analyses of the data on survival, duration, rate of molting, mortality and behavior are presented.

This Project Summary was developed by EPA's Environmental Research Laboratory, Gulf Breeze, FL, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Part I—Effects of Soluble Fractions of Used Light-Weight Lignosulfonate Type Mud on the Complete Larval Development of Crabs, *Rhithropanopeus harrisii* and *Callinectes sapidus*

Introduction

The Jay Exxon well drilling fluid 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 U.S. 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 of offshore wells.

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 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 it contains a higher concentration of particulates and a lower concentration of volatiles. These two fractions are found in the upper plume of discharge and remain in the water column longer than other fractions, and, hence, may be the fractions which might be expected to affect larvae of marine organisms as well as plankton.

At present, the impact of drilling fluids is incompletely known. Most investigations have been acute toxicity tests. 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. In this investigation, chronic tests were employed because a study covering the entire larval development of crabs would give a better evaluation of possible toxicity of drilling fluids in the field than an 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, it is possible to determine which stage or stages in the larval development are particularly sensitive if mortality is recorded at each stage of development.

Results and Conclusions

The percent survival to megalopa and to 1st crab stage of *Rhithropanopeus harrisii* was 90% or over in seawater control and in all concentrations of MAF and SPP. There was no consistent reduction in survival in concentrations of MAF and SPP compared to survival in seawater control. Hence, 100% MAF and 100% SPP light-weight lignosulfonate type mud is non-toxic to developing larvae of *R. harrisii* throughout complete larval development.

The average percent survival of three series of *Callinectes sapidus* reared in seawater control and four concentrations of MAF and SPP is listed in Table 1. 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 control. There was differential survival, however, from 5% MAF and SPP to 100% MAF and SPP.

Statistical analysis revealed for *C. sapidus* zoeal survival to megalopa that there was approximately 4% decrease/10% increase in MAF @ 50% CONC., and for survival to 1st crab, there is approximately 3% decrease/10% increase in MAF @ 50% CONC. For *C. sapidus* zoeal survival to megalopa and for survival to 1st crab, there was approximately a 5% decrease in survival for a 10% increase in SPP near 50% SPP CONC.

From Table 1 it can be observed that there was no significant difference in duration in zoeal development of *C. sapidus* and in hatch to 1st crab in seawater control and in the concentrations of MAF and SPP employed.

The results illustrated in Figure 1 show the effect of MAF levels on the mortality of larvae at each of the seven to eight zoeal stages and a megalopa stage through which *C. sapidus* passes. 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, 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 1).

Mortality of *C. sapidus* 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 (Figure 2). For zoeal stage I, mortality in 25% SPP was significantly different from the control at the 0.05 level. As in the MAF experiment, zoeal stages I and II of *C. sapidus* were the most sensitive in all media. In general, SPP was more toxic to blue crab larvae than MAF.

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 in speed was only observed in 100% MAF, but it was found in all percentages of SPP tested.

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 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.

Table 1. *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	–	–	–

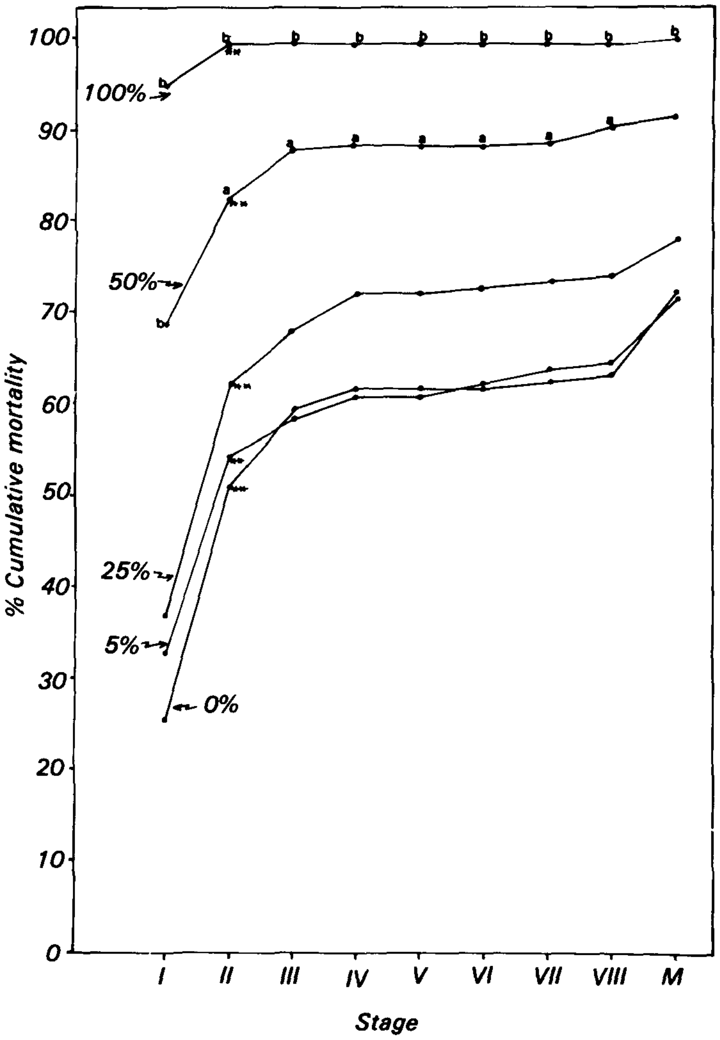

Figure 1. *Effect of MAF of used light-weight lignosulfonate type mud on mortality by stages of development of* C. sapidus.

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)*

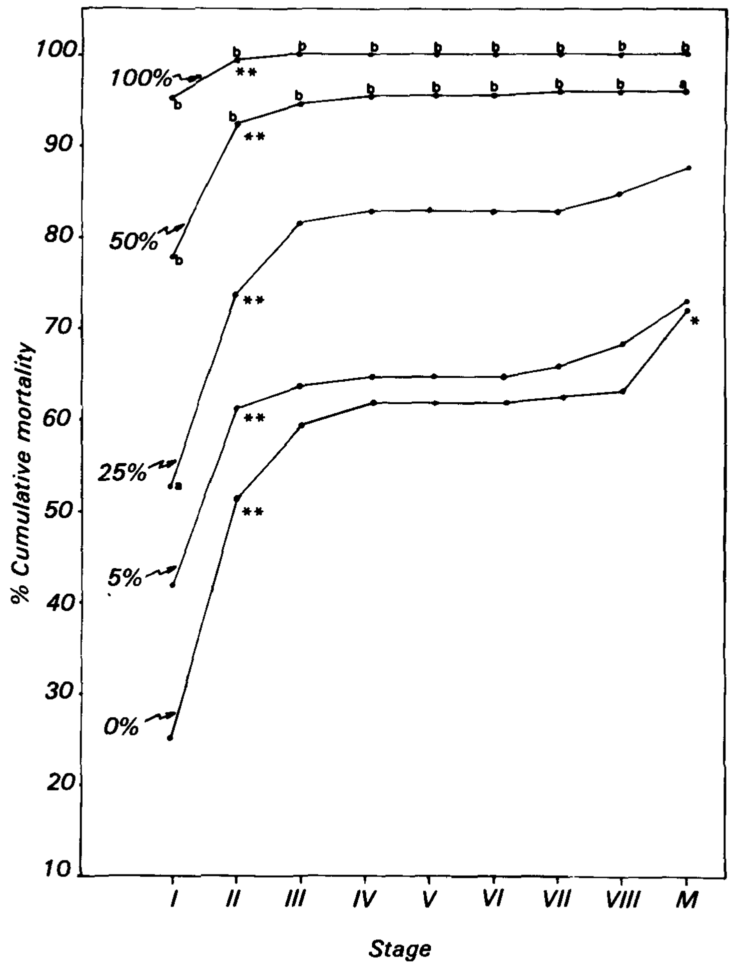

Figure 2. *Effect of SPP of used light-weight lignosulfonate type mud on mortality by stages of development of* C. sapidus.

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)*

Part II—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 varies with valence state, pH and oxidation states. Hexavalent chromium (Cr^{+6}) is stable in seawater. It often appears as a soluble chromate or dichromate and both are 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 acid 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. 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. Ferrochrome lignosulfonate, brandname "Q-Broxin," and chrome lignosulfonate are common additives to drilling fluids which contribute to Cr enrichment. Initially, these additives contain hexavalent salts, but at temperatures between 120 and 175 °C hexavalent chromium is converted to the trivalent state. The properties of both of these additives can be restored at temperatures between 120 and 175 °C by adding more Cr^{+6}.

It has 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. Once freed from clay particles, 3 to 7% Cr^{+3} through slow oxidation may revert to Cr^{+6}.

From the above discussion, we conclude that under certain conditions the possibility exists that both trivalent and hexavalent chromium may be in the vicinity of the discharge source.

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.*

Results and Conclusions

In experiments on the effect of hexavalent chromium, Na_2CrO_4, on the development of *Rhithropanopeus harrisii*, it was observed that there were no significant differences between survival from hatching to megalopa and hatching to 1st crab in seawater control and 1.1 ppm Na_2CrO_4, but there was differential survival from 1.1 to 29.1 ppm Na_2CrO_4 (Table 2). The estimated LC50 for zoeal development from hatching to megalopa was 17.8 ppm Na_2CrO_4, and from hatching to 1st crab, the estimated LC50 was 13.7 ppm Na_2CrO_4.

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

From Figure 3, it can be seen that 1 ppm Na_2CrO_4 is nontoxic to *R. harrisii* larvae, for there is no more mortality in this concentration than in seawater control. There is differential larval mortality from concentrations of 1.1 ppm to 58.1 ppm Na_2CrO_4. Concentrations of 7.2 ppm and 14.5 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 Na_2CrO_4 and none reached the 1st crab stage in 41, 46 and 58 ppm Na_2CrO_4.

Rhithropanopeus larval swimming speed was affected by exposure to Na_2CrO_4. In general, low sublethal concentrations caused an increase in swimming speed and concentrations near those which are acutely toxic caused a decline.

In experiments on the effect of hexavalent chromium, Na_2CrO_4, on the development of *Callinectes sapidus*, it was observed that survival from hatching to 1st crab occurred in 1.1 to 4.7 ppm Na_2CrO_4 (Table 3). There was better survival in 1.1 ppm Na_2CrO_4 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 of *C. sapidus* was estimated to be 2.9 ppm Na_2CrO_4, and the LC50 for development from hatching to 1st crab stage was estimated to be 1.0 ppm Na_2CrO_4.

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.

From Figure 4, it can be seen that there is significantly less mortality of *C. sapidus* larvae in 1.1 ppm Na_2CrO_4 than in seawater control. There is also less larval 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 of *C. sapidus* larvae 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. Blue crab larvae in zoeal stage III were extremely sensitive to 7.2 ppm Na_2CrO_4, and larvae in zoeal stages III, IV and V were most sensitive in 4.7 ppm Na_2CrO_4.

For most discharges, the background concentration for chromium has been reported to be reached approximately 100 to 150 meters from the point of discharge, depending on the amount and the rate of discharge, as well as the currents. 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 ques-

Table 2. *Average Percent Survival and Average Duration in Days of Zoeal and Megalopa Development of* R. harrisii *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 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			

tionable, 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.

Table 3. *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	–	–	–	–	–

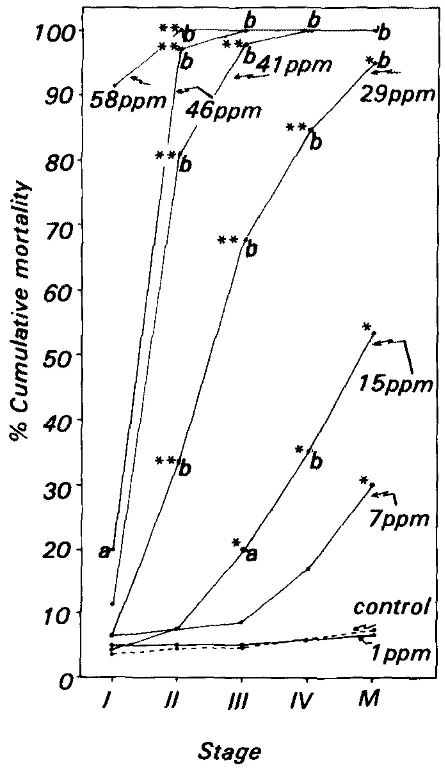

Figure 3. **Effect of Na_2CrO_4 in ppm on mortality of *R. harrisii* larvae.**
- a. Significantly different from control (0.05)
- b. Significantly different from control (0.01)
- *. Significant increase over previous stage (0.05)
- **. Significant increase over previous stage (0.01)

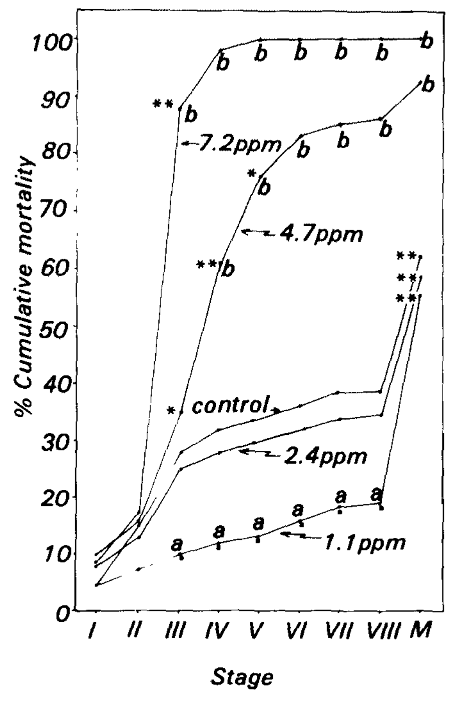

Figure 4. **Effect of Na_2CrO_4 in ppm on mortality of *C. sapidus* larvae.**
- a. Significantly different from control (0.05)
- b. Significantly different from control (0.01)
- *. Significant increase over previous stage (0.05)
- **. Significant increase over previous stage (0.01)

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The complete report, entitled "Effects of Soluble Fractions of Drilling Fluids and Hexavalent Chromium on the Development of the Crabs, Rhithropanopeus harrissi *and* Callinectes sapidus,*" (Order No. PB 82-197 203; Cost: $9.00, subject to change) will be available only from:*
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