INFLUENCE OF DISSOLVED HUMIC MATERIALS ON THE BIOLOGICAL EFFECTS OF DRILLING COMPONENTS

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Introduction

In recent years, concern has grown that land-based drilling wastes may be harmful to terrestrial and freshwater ecosystems. These wastes may include drilling fluids and cuttings along with large quantities of brine. As much as 10,000 to 40,000 barrels (bbl) of drilling fluids may be discharged during the operation of a single well (Neff 1980). Drilling fluids, or "muds", perform various functions in the drilling process, such as cooling and lubricating the drill bit, removing drill cuttings, controlling formation pressures, stabilizing the borehole, and controlling corrosion (Szmant-Froelich 1983). These drilling fluids are a complex mixture of clays and barite, as well as a series of chemical and specialty additives which may vary to suit drilling conditions (Duke and Parrish 1984). Chemical additives enhance the properties of drilling fluids and give the fluids characteristics necessary to alleviate problems peculiar to each drilling operation. Chrome lignosulfonates (Cr Lig) are common chemical additives used as deflocculents for thinning water-based drilling fluids, in addition to maintaining the mud in a fluid state (U.S. EPA 1985a). Specialty additives are used in relatively small quantities; however, they may disproportionately contribute to the toxicity of drilling fluids. The same holds true for process contaminants of drilling fluid discharges, which contain many toxic trace metals, such as arsenic, cadmium, chromium, lead and zinc (Houghton et al. 1984).

The potential toxicity of, and surface and ground water contamination by, trace metals associated with drilling fluids is of environmental concern. Although many states now have legislation regulating the disposal of drilling fluid wastes generated during inland operations, there are numerous abandoned waste pits nationwide. For example, in Louisiana alone there are over 13,000 abandoned drilling fluid pits (Petzinger and Getschow 1984). While there is considerable information available on the environmental fate and effects of drilling discharges from offshore, or marine, operations, there is a paucity of data concerning their impact on freshwater ecosystems.

The ability to accurately predict the toxicity of trace metals associated with drilling fluids in the freshwater ecosystem is dependent on understanding the behavior of these elements in the environment. However, trace metals in natural waters often do not react as predicted by the known chemistry of the metal in question. This paradox is due, in part, to the interaction of metals with both inorganic and organic constituents of the freshwater chemical matrix. Dissolved humic materials (DHMs) constitute the major portion, 60 to 80%, of the total dissolved organic material (DOM) in surface waters, sediments and soils (Boggs et al. 1985). DHMs exist as high-molecular-weight heterogeneous complexes, with molecular weights of up to 300,000 daltons (Stewart 1984). In addition, DHMs are the most important type of DOC because of their ability to form stable complexes with metal ions (Boggs et al. 1985). Natural aqueous concentrations of DHM range from 1 to 70 mg/l, with a worldwide average of 5.8 mg/L (Boggs et al. 1985). The higher concentrations of DHM (50 to 70 mg/L) represents black-water systems such as bogs, marshes and swamps (Stewart 1984).

Dissolved humic materials appear to have contradictory effects on the bioaccumulation of trace metals by marine and freshwater organisms. In the marine bivalve Mytilus edulis, the uptake and tissue accumulation of cadmium was increased by DHM (George and Coombs 1977). However, DHM decreased the tissue accumulation of cadmium in another marine bivalve, Crassostrea virginica (Hung 1982). In freshwater organisms, DHM has been

observed to decrease, increase, or have no effect on the bioaccumulation of trace metals. Cadmium bioaccumulation was decreased in Daphnia magna (Poldoski 1979) and Selenastrum capricornutum (Sedlacek et al. 1983) in the presence of DHM. In addition, DHM decreased the bioaccumulation of copper by Simocephalus serrulatus (Giesy et al. 1983). In hard water, copper bioaccumulation was significantly increased by DHM in D. magna, although there was no effect observed in soft and medium hard water (Winner and Gauss 1986). In general, DHM had no influence on the bioaccumulation of zinc and cadmium by D. magna (Winner 1984; Winner 1986; Winner and Gauss 1896).

In view of the contradictory nature of the available data, the objective of the present investigation was to evaluate the influence of DHM, humic acid (HA), on the toxicity and bioaccumulation of selected trace metals associated with drilling fluid components, cadmium and chromium. Chromium (Cr) was tested as hexavalent chromium (Cr VI) and two forms of trivalent chromium (Cr III), chromic chloride ($CrCl_3$) and Cr Lig. Cr Lig is a form of Cr III used as a deflocculent to thin water-based drilling fluids, in addition to maintaining the fluidity of the drilling fluids (U.S. EPA 1985a). Although considerable information is available on the toxicological effects of drilling fluid components, such as Cr Lig, on the marine environment, there are few data concerning their impact on freshwater systems. Therefore, Cr Lig was used as a model compound to improve our understanding of the environmental behavior of drilling fluid components in freshwater ecosystems.

Materials And Methods

Culture and Test Conditions

Due to their ecological significance, the cladocerans Daphnia pulex and D. magna were selected as animal models for this investigation. Cladocerans are an integral part of the freshwater food chain and often comprise 70 to 90% of the diets of young fish (Pennak 1978). In addition, daphnids offer advantages of small size, short life span, ease of culture, genetic uniformity, and sensitivity to water-borne contaminants. The original laboratory cultures of D. pulex and D. magna were obtained from the U.S. Environmental Research Laboratory, Environmental Research Laboratory in Duluth, MN, and the University of Texas at Dallas, respectively. The daphnids were cultured using green algae, Selenastrum capricornutum and cerophyll, yeast, and trout food mixture (Stackhouse and Benson 1988; 1989).

The acute toxicity bioassays were conducted in reconstituted moderately hard water (U.S. EPA 1985b). The HA (Aldrich Chemical Co.) was prepared by dissolution and centrifugation at 6,000 g for 30 min, and then filtration through a 1.2 µm membrane filter to remove particulates. Cadmium (Cd) and Cr VI were delivered in the forms $CdCl_2 \cdot 2\ 1/2\ H_20$ and $K_2Cr_2O_7$, respectively. Cr III was delivered as both $CrCl_3 \cdot 6\ H_2O$ and Cr Lig. Cr Lig was supplied by IM Drilling Fluids (Houston, TX). The pH of the stock solutions of both Cr II forms was adjusted with NaOH to 8.0. All metal stock solutions were prepared in dilution water.

Acute Toxicity Bioassays

Acute toxicity of the trace metals was determined by median lethal concentration (LC_{50}) bioassays using 24-h old or less D. pulex neonates. Twenty neonates were placed in 400 ml glass beakers containing 200 ml of varying concentrations of metal (at least 5 concentrations and a control). Due to precipitation, the acute toxicity of $CrCl_3$ was determined using static-renewal bioassays with metal solutions renewed every 24 h. The remaining trace metals were tested under static conditions. The LC_{50} of each metal was determined at 24, 48, 72 and 96 h. The influence of HA on acute toxicity of the metals was then assessed with the addition of HA to the dilution water. Final concentrations of HA tested were 0, 0.5, 5.0 and 50 mg/L. Concentrations of 1 to 50 mg/L DOM often occurs in most lakes and streams (Stewart 1984). The 0.5 mg HA/L concentration represented a pristine, but yet natural, water source. The LC_{50} values of each metal, in the presence of HA, was calculated for the same time points as the HA control.

Bioaccumulation

The effect of HA on the bioaccumulation of trace metals was determined by a modified version of Winner (1984). Forty D. magna (7 d old) were exposed in 1,000-ml glass beakers containing 800 ml solution. The dilution water contained 10 µg/L Cd or the various Cr species and one of four HA concentrations (0, 0.5, 5.0 and 50 mg/L). Seven-day-old D. magna were used to provide a greater biomass for the acid-extraction of trace metals. Test solutions were renewed every 24 h and the daphnids were fed 1.25 mg dry wt./L S. capricornutum at each renewal. Prior to and following renewal, water quality

characteristics were measured. At selected time points (1, 3, 6, 12, 24, 48, 72, and 96 h), daphnids were transferred through two rinses of deionized, distilled water, dried at 70 °C and weighed in groups of 10 on a Mettler M5 microbalance. After weighing, the daphnids were prepared for total metal analysis by a modified version of the acid-extraction procedure of Benson et al. (1983). Prior to metal analysis, Daphnia were predigested at room temperature with 0.1 ml H_2NO_3 (Baker Instra-Analyzed) in test tubes with Teflon-lined screw caps. After 24 h, the samples were heated in a waterbath at 70 °C for 6 h. Following digestion, each tube was brought up to 1 ml with deionized, distilled water, and stored at 4 °C until analyzed for metal content.

Chemical Analysis

Aqueous and tissue metal concentrations were determined using a Perkin-Elmer atomic absorption spectrophotometer (Model 3030B) equipped with a graphite furnace (Model HGA-600) by the modified methods of Arpadjan and Krivan (1986). The detection limits for cadmium and chromium were 0.5 and 1 µg/L, respectively. The experimental concentrations of HA were determined by the UV spectrophotometric method of Zitko et al. (1973) using a Gilford Response UV-VIS spectrophotometer. The detection limit for HA was 0.4 mg/L.

Data Analysis

Median lethal concentration (LC_{50}) values and 95% confidence limits were calculated by probit analysis (SAS Institute Inc. 1985). Significant differences between LC_{50} values obtained from the bioassays, were assessed by the standard error of the difference (Sprague and Fogels 1977). Treatment effects were evaluated using one-way analysis of variance. Means separation of data was achieved by Scheffe's test (SPSS Inc. 1983). For nonhomogeneous data, square root transformations achieved homogeneity. The Dixon test was used to reject outliers obtained in the bioaccumulation studies (Taylor 1987). Statistical differences were considered significant at $p<0.05$.

Results

Results from acute toxicity bioassays will be discussed in context to results obtained from bioaccumulation studies. These toxicity data, however, have been previously published (Stackhouse and Benson 1988; 1989).

Humic acid had little influence on the bioaccumulation of Cr VI by D. magna (Table 1). The mean background level of Cr in the daphnids was 4.0 µg/g. At time points 1 through 72 h Cr content increased and HA had no significant effect on uptake. By 96 h, however, 50 mg/L HA did significantly decrease the bioaccumulation of Cr VI, while the remaining HA concentrations (0.5 and 5.0 mg/L) continued to have no influence on bioaccumulation.

Table 1: Influence of humic acid (HA) on the bioaccumulation (µg Cr/g dry wt.) of 10 µg/L hexavalent chromium by Daphnia magna

Exposure period (h)	HA concentration (mg/L) 0	0.5	5.0	50.0
0	4.0	-	-	-
1	6.5	6.7	4.5	7.7
3	9.4	8.8	7.8	7.7
6	13.7	16.9	17.0	12.4
12	33.5	41.0	37.5	37.5
24	48.9	43.4	46.8	32.9
48	41.9	40.4	50.3	42.3
72	64.2	75.4	62.5	54.0
96	65.8	58.5	67.8	52.1*

Values are means (n = 3-4).
* Significantly different ($p<0.05$) from simultaneous control (0 mg HA/L).

Humic acid had a greater impact on the bioaccumulation of $CrCl_3$ than Cr VI (Table 2). Daphnid Cr content increased throughout the exposure to $CrCl_3$ in the presence of 0 and 0.5 mg/L HA. However, Cr content did not consistently increase at 5 and 50 mg/L HA. As with $CrCl_3$, HA had a significant influence on the bioaccumulation of Cr Lig (Table 3).

Table 2: Influence of humic acid (HA) on the bioaccumulation (µg Cr/g dry wt.) of 10 µg/L chromic chloride by Daphnia magna

Exposure period (h)	HA concentration (mg/L) 0	0.5	5.0	50.0
0	4.0	-	-	-
1	15.1	14.0	17.8	9.3
3	27.4	23.7	17.9*	8.7*
6	35.7	21.8	24.6	8.0*
12	54.2	43.4	49.7	34.0
24	62.4	74.8	46.3	28.1*
48	80.3	73.8	53.7	20.1*
72	83.8	84.6	41.4*	14.1*
96	105.8	100.8	64.2*	32.8*

Values are means (n = 3-4).
* Significantly different ($p<0.05$) from simultaneous control (0 mg HA/L).

Table 3: Influence of humic acid (HA) on the bioaccumulation (µg Cr/g dry wt.) of 10 µg/L chrome lignosulfonate by Daphnia magna

Exposure period (h)	HA concentration (mg/L) 0	0.5	5.0	50.0
0	4.0	-	-	-
1	14.0	10.7	5.7	3.3
3	9.0	8.3	4.5	1.8*
6	12.8	7.4*	6.2*	2.6*
12	56.8	40.1	25.1	11.4*
24	20.4	15.0	13.7*	4.0*
48	40.3	39.3	18.3	8.2*
72	55.5	37.8	25.0	17.0*
96	44.1	24.1	12.9	9.3*

Values are means (n = 3-4). * Significantly different ($p<0.05$) from simultaneous control (0 mg HA/L).

Although a variety of effects were observed on Cd bioaccumulation, there was a general trend toward decreased Cd uptake in the presence of HA (Table 4). The mean background concentration in Daphnia was 2.6 µg/g. Cd content increased at all time points at all HA concentrations.

Table 4: Influence of Humic acid (HA) on the bioaccumulation (µg Cd/g dry wt.) of 10 µg/L cadmium by Daphnia magna

Exposure period (h)	HA concentration (mg/L) 0	0.5	5.0	50.0
0	2.6	-	-	-
1	7.3	6.9	7.4	5.0*
3	7.0	11.0*	12.7*	5.8
6	12.8	15.0	14.3	8.8*
12	15.5	16.8	16.3	11.6*
24	20.2	17.3	19.3	12.4*
48	30.8	34.2	28.6	15.7*
72	43.1	39.6	37.2	18.0*
96	65.0	57.8	49.5*	24.6*

Values are means (n = 3-4).
* Significantly different ($p<0.05$) from simultaneous control (0 mg HA/L).

Discussion

HA had a greater influence on the bioaccumulation of Cd and the Cr III compounds than of Cr VI. The 50 mg/L HA concentration significantly decreased the bioaccumulation of Cd and both Cr III compounds ($CrCl_3$ and Cr Lig) at most of the time points examined, while only significantly decreasing bioaccumulation of Cr VI by 96 h. In addition, 5 mg/L HA also decreased bioaccumulation of Cd and $CrCl_3$ by 96 h and 72 h, respectively. These results agree with acute toxicity and bioavailability data previously obtained in our laboratory (Stackhouse and Benson 1988; 1989). The toxicity of Cd and Cr III compounds was significantly decreased by 50 mg/L HA at all time points examined, while Cr VI toxicity was significantly decreased only by 48 h. The 50 mg/L HA also decreased the bioavailability (or percent free metal) of Cd and $CrCl_3$ to a greater extent than it did that of Cr VI. These results are consistent with the findings of the U.S. Environmental Protection Agency (U.S. EPA

1980a; 1980b), which indicated that, in general, Cd and Cr III are significantly influenced by inorganic and organic compounds, while Cr VI is not.

The most likely explanation for the metal-specific results obtained in the present investigation relates to the speciation of trace metals in freshwater. For the dilution water used in this investigation, the thermodynamic data would indicate that the dominant dissolved species would be Cd^{2+} for Cd, CrO_4^{2-} for Cr VI and $Cr(OH)^{2+}$ for Cr III (Cranston and Murray 1980; Mantuora et al. 1978). Trace metals generally bind to HA by proton removal from carboxylic acid or hydroxyl functional groups followed by coordinate bonding (Giesy and Alberts 1982). This complexation mechanism indicates that HA would bind readily to a cation (e.g., Cd and Cr III) but not to an anion (e.g., Cr VI). However, anionic compounds will bind weakly to HA through hydrogen bonding (Stevenson 1972).

It is interesting to note that $CrCl_3$ was bioaccumulated to a significantly greater extent than a comparable level of Cr VI. In the presence of 0 mg/L HA, the daphnids accumulated 105.8 µg Cr/g of $CrCl_3$ by 96 h, whereas the Cr VI was accumulated to only 65.8 µg Cr/g. These findings would seem to indicate that there was a mechanism for the selective uptake of Cr III. A possible explanation for these results is that Cr III is an essential micronutrient (Mertz 1969). Cr III is utilized by most organisms for the maintenance of normal glucose tolerance (Arpadjan and Krivan 1986) and it may be more readily absorbed from the aqueous environment.

The slight increase in bioaccumulation of Cd in the presence of low concentrations of HA has previously been reported in marine organisms (George and Coombs 1977). In freshwater organisms, Copper bioaccumulation also has been observed to be increased in the presence of HA (Winner and Gauss 1986), whereas HA has been demonstrated to only decrease or not influence Cd bioaccumulation (Poldoski 1979; Sedlacek et al. 1983; Winner 1984; Winner 1986; Winner and Gauss 1986). As a possible explanation for the increased bioaccumulation, George and Coombs (1977) proposed that the complexed form of Cd would be in more rapid equilibrium with the ligands of the cell membrane than cadmium chloride, therefore facilitating entry into the cell. This increase in Cd bioaccumulation could possibly explain the increase in toxicity of the metal of Daphnia pulex in the presence of 0.5 mg/L HA observed in earlier investigations (Stackhouse and Benson 1988). With the exception of tolerance studies in which inverse correlations between whole-body accumulation and toxicity have been demonstrated (Brown 1977; Dixon and Sprague 1981), it is generally accepted that an increase in metal accumulation results in increased toxicity. Therefore, an HA-induced increase in Cd bioaccumulation by the daphnids would lead to a greater degree of lethality.

Conclusions

The interaction of trace metals and DOM, such as HA, can result in an altered physicochemical form of the metal, which in turn, may affect bioaccumulation. The results of this investigation indicate that the influence of HA on the bioaccumulation of Cr and Cd is dependent on (a) oxidation state, (b) temporal relationship and (c) HA concentration.

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