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NATURE AND EXTENT OF THE INTERACTIONS OF HUMIC ACIDS WITH A WATER TREATMENT ALGICIDE AND A FUNGICIDE

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ABSTRACT

Equilibrium dialysis and molecular sieve chromatography were employed to evaluate the interactions between humic acids (HA) and a water treatment algicide, poly[oxyethylene(dimethyliminio)ethylene dimethyliminio)ethylene dichloride (WSCP), and a fungicide, 2-(thiocyanomethylthio)benzothiazole (TCMTB). WSCP exhibited saturable, reversible binding to HA. The interaction between HA and WSCP was found to be of sufficient magnitude that naturally occurring HA concentrations will have significant impacts on the bioavailabity, toxicity and fate of WSCP in the environment. No interaction between HA and TCMTB was detected.

INTRODUCTION

The ability of water resource managers to accurately predict the fate and toxicity of chemicals in aquatic ecosystems is essential for the development of reliable, site-specific water quality standards. The HA components of naturally occurring dissolved organic material in aquatic ecosystems have been shown to be important in influencing these parameters. The purpose of this investigation was to evaluate the nature and extent of binding interactions between HA and two chemicals, WSCP and TCMTB. WSCP (poly[oxyethylene(dimethyliminio)ethylene(dimethyl-iminio)ethylene dichloride]), a water treatment algicide, is highly water soluble and has an average molecular weight of 3900 ranging from approximately 600 to 5000. TCMTB (2-(thiocyanomethylthio)benzothiazole), molecular weight 206, is a fungicide of limited water solubility. Because of their intended uses these chemicals will be transported into aquatic environments.

Humic materials, which originate from microbial degradation and polymerization of plant materials, constitute 60% to 80% of the dissolved organic content of surface waters (1). Dissolved humic materials (DHM) are, in general, polyphenolic compounds with multiple carboxyl, carbohydrate and peptide moieties (2). Natural aqueous concentrations

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of DHM range from 1 to 70 mg/L, with a worldwide average of 5.8 mg/L (1). The higher concentrations of DHM (50 to 70 mg/L) represent "blackwater systems" such as bogs, marshes and swamps (2).

Dissolved humic materials are composed of humic and fulvic acids (1). Aquatic HA, which have typical average molecular weights of 2,000 to 3,000, are soluble in alkaline conditions but precipitate in acidic conditions. Aquatic fulvic acids have molecular weights of less than 1,000 and are soluble in both acids and bases (3-5).

Complexation of a variety of organic chemicals with DHM has been shown to affect chemical bioavailability and toxicity in addition to influencing the breakdown and mobility of chemicals in the environment. For example, Chiou et al (6) demonstrated water solubility enhancements of polychlorinated biphenyls and DDT by DHM as well as by commercially available HA. It has been demonstrated that DHM reduced the bioconcentration of polycyclic aromatic hydrocarbons by the freshwater cladoceran, *Daphnia magna* (7) and bluegill sunfish (8). Carlberg et al. (9) showed that natural water, containing humic materials, reduced the bioavailability of selected trichlorophenols and lindane. Stewart (2) reported that DHM enhanced the toxicity of *o*-cresol, 2,4-dimethylphenol, and 2,3,6-trimethylphenol while reducing the toxicity of *p*-benzoquinone and quinoline. Carter and Suffet (10) demonstrated that HA showed a greater tendency to bind polycyclic aromatic hydrocarbons and pesticides than fulvic acids. Previous research in our laboratories (11-13) has shown that HA interact with some pesticides resulting in significant changes in their toxicities, whereas the remainder of the aquatic organic carbon does not significantly alter the toxicities of such pesticides at environmentally relevant concentrations.

There is evidence to suggest that the toxicity of WSCP to freshwater organisms is influenced by the presence of humic materials (14). In acute tests with bluegill sunfish, the LC_{50} value of WSCP in laboratory water was 0.34 mg/L. When exposed in natural river water, containing humic materials, the LC_{50} value increased to 6.70 mg/L. Similarly, the LC_{50} of WSCP for *Daphnia magna* was increased from 0.60 mg/L to greater than 7.50 mg/L in water with 10 mg/L HA.

TCMTB has been shown to bind more strongly to soils with higher organic content (15). These findings suggest that TCMTB may bind to HA. However, no binding of TCMTB to creek sediment containing 6% organic carbon was detected and TCMTB solubility was not affected by creek water that contained 8.2 mg/L of dissolved organic carbon (16)

EXPERIMENTAL

Materials

WSCP, [^{14}C]-WSCP (radiolabelled by Sigma Chemical Co., St. Louis, MO), TCMTB, [^{14}C]-TCMTB (radiolabelled by ABC labs., Columbia, MO), were provided by Buckman Laboratories, International, Memphis, TN. Sephadex^{R} G-150-120 was obtained from Sigma Chemical Co., St. Louis, MO. The HA employed for this investigation were the acid precipitable fraction of Aldrich^{R} HA (Aldrich Chemical Co., Milwaukee, WI). These were sized by gel filtration through Sephadex^{R} G-150-120 as described below.

Methods

All of the experiments were performed at neutral pH (6.5 to 7.5) and at ambient temperature (23°C to 26°C) Attempts were made to measure binding of WSCP to both high (10,000-150,000) and low (1,000-10,000) molecular weight HA employing equilibrium dialysis and ultracentrifugation techniques. As outlined in the results section these methods were unsuccessful.

Binding of WSCP to low molecular weight HA was measured by the gel filtration technique of Hummel and Dreyer (17). Sephadex^{R} G-150-120 was hydrated in water containing 1 x 10^{-4} M NaN_3 as a bacteriostat and packed into columns 95 cm high by 0.75 cm in diameter. Identical columns were used for sizing the HA. Molecular weight ranges were determined using the elution volumes of protein and polypeptide molecular weight standards (M.C. Kadlec, unpublished data). Columns were equilibrated with [^{14}C]-WSCP under gravity flow at a hydrostatic pressure head of 15 cm and a flow rate of approximately 2 ml/hr. Then 1 ml aliquots of HA in the [^{14}C]-WSCP solution were passed through the columns. As the HA passed through the column it picked up excess WSCP due to binding interactions. The excess WSCP over base line, which eluted with the HA, was taken as the bound WSCP. [^{14}C]-WSCP was quantified by liquid scintillation counting and HA was quantified by measuring the absorbance at 260 nm. The standard deviation (S.D.) for the baseline readings of [^{14}C]-WSCP was 9.3 cpm and the standard deviation for baseline readings of HA absorbance at 260 nm was 0.0008 absorbance units. Any readings that were greater than 3 S.D. above baseline were considered to be significantly different from baseline, and 3 S.D. above baseline was taken as our detection limit for bound WSCP.

TCMTB interaction with HA was assessed using equilibrium dialysis. Solutions of HA, 10,000 to 150,000 molecular weight, were placed in dialysis bags (Spectrapor^{R}, Los Angeles, CA, 10,000 to 12,000 molecular weight cut off) and these were suspended in [^{14}C]-TCMTB solutions. These were incubated for at least 24 hr to achieve equilibrium. HA concentrations ranged from 3 mg/L to 1000 mg/L and equilibrium concentrations of TCMTB ranged from 0.002 mg/L to 3.5 mg/L. Controls were also run with no HA in the dialysis bags.

Data for WSCP binding to HA were analyzed by nonlinear fitting to the Langmuir binding isotherm:

$$B = \frac{B_{max}\,[WSCP]}{K_D + [WSCP]}$$

employing NLIN^{R} software (V. Singh, Cranford, NJ). B is the amount of WSCP bound in mg/L, B_{max} is the maximum possible amount of WSCP that can bind to the HA present, K_D is the equilibrium binding constant for the interaction between HA and WSCP, and [WSCP] is the unbound concentration of WSCP in mg/L.

RESULTS

Several alternative methods (including equilibrium dialysis and ultracentrifugation) for measuring the binding of WSCP to HA were examined. Only the gel filtration technique with low molecular weight HA provided interpretable data. High molecular weight HA forms insoluble aggregates with WSCP. When high molecular

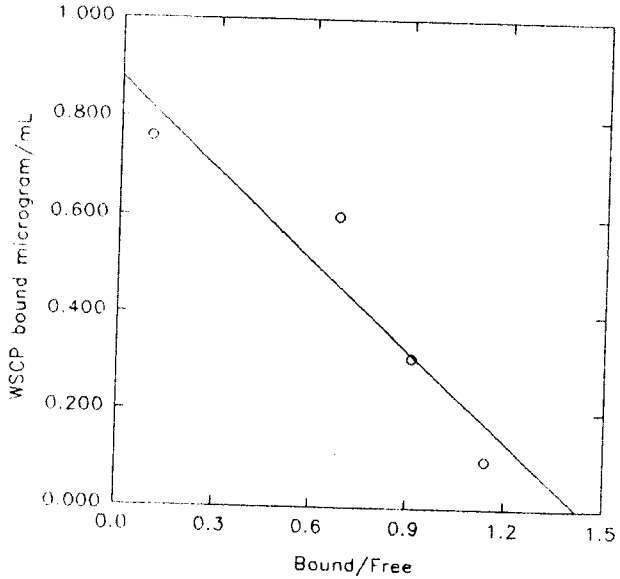

Figure 1. Eadie-Hofstee plot of data from Table 1 on the binding interaction between low molecular weight HA and WSCP.

weight HA concentrations are in the range from 1 to 30 mg/L the optimal ratio for this insoluble aggregate formation is 3 to 3.5 mg of HA per mg of WSCP. High molecular weight HA molecules apparently have multiple binding sites for WSCP, which, in turn, have multiple binding sites for HA. This gives rise to the possibility for formation of large complexes. The ratio of components is the most important consideration for determining the amount of insoluble complex. Too much of either component will saturate the binding sites on the other component and limit the size of the complex. Since mixtures of high molecular weight HA and WSCP contain both soluble and insoluble complexes we could not employ the soluble portion as the unbound fraction.

Other attempts to separate bound from unbound WSCP were thwarted due to the fact that WSCP binds to glass, siliconized glass, several kinds of plastic, Teflon^{R}, aluminum, tin and stainless steel at least as well as it binds to HA. WSCP also binds very strongly and with high capacity to dialysis membrane and Sephadex^{R} gel. Problems with the gel filtration method were overcome by equilibrating the column with WSCP and only measuring the solution levels of WSCP and HA in the column eluates. Thus, any insoluble complexes, which may have formed, and any binding to column components became irrelevant. We did have some error due to binding of WSCP to the collection tubes and transfer pipettes. This error was considered to be relatively small and constant and would have the effect of underestimating the binding of WSCP to HA.

Sufficient data were collected at different HA and WSCP concentrations (Table 1) to generate the Eadie-Hofstee plot shown in Figure 1. Figure 2 is a typical elution profile for the column. WSCP forms a soluble complex

Table 1. Chromatographic data for interaction of low molecular weight HA and WSCP

Baseline [WSCP] (Free) mg/ml	Peak [HA] mg/ml	Peak [WSCP] (Bound) mg/ml	Bound/Free
0.000088	0.0030	0.00010	1.14
0.00034	0.0040	0.00031	0.91
0.00088	0.0038	0.00060	0.68
0.0080	0.0046	0.00076	0.095

with low molecular weight HA with maximum binding capacity (B_{max}) of 0.225 mg WSCP/mg HA and dissociation constant (K_D) of 6.4 mg/L. The binding interaction between WSCP and low molecular weight HA is saturable, reversible, and gives a reasonable fit to the Langmuir binding isotherm. The B_{max} value indicates that the molar binding ratio of WSCP to low molecular weight HA is approximately one to one at saturation. The dissociation constant indicates that one half of the binding sites for WSCP on the available HA will be occupied when the WSCP concentration is 6.4 mg/L (6.4 ppm).

There was no detectable binding of TCMTB to HA, under any of the conditions employed. These ranged from minimum detectable levels of ^{14}C labelled TCMTB to nearly one tenth of the solubility limit of TCMTB in water (16) and a relatively wide range of HA concentrations.

DISCUSSION

According to the manufacturer's instructions 200 ppm is the upper treatment level for WSCP. Therefore, it is unlikely that environmental levels higher than this will occur from recommended usage. At this level the average HA concentration of 5 mg/L will bind approximately 0.5% of the WSCP. It is more likely that environmental concentrations of WSCP will be substantially less than 200 ppm. At 6.4 ppm, 8.8% of the WSCP will be bound to HA when the HA concentration is 5 mg/L, at 0.64 ppm 16% of the WSCP will be bound to HA when the HA concentration is 5 mg/L, and at 0.064 ppm 17% of the WSCP will be bound to HA when the HA concentration is 5 mg/L. These numbers indicate that between 10% and 20% of WSCP may be bound in soluble complexes to naturally occurring HA under expected levels of WSCP contamination in aquatic ecosystems. This level does not appear to be high enough to explain the effects of DHM and HA on WSCP toxicities quoted in the introduction. It must be kept in mind, however, that WSCP also forms insoluble, highly stable complexes with HA and these are expected to represent a substantial contribution to the removal of WSCP from the water column.

Numerous investigators have published partition coefficients for the interaction between various toxic and hazardous substances with HA. Some of these values, which were determined for the acid precipitable fraction of Aldrich^{R} HA, are given in Table 2 along with the literature source from which they were obtained. A partition

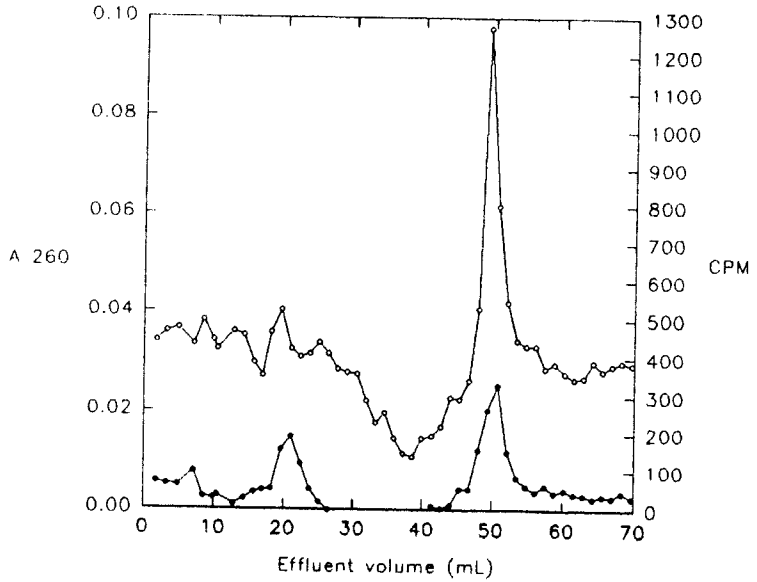

Figure 2. Representative Sephadex[R] G-150-120 column elution profile for the interaction of low molecular weight HA with WSCP. The open circles represent the values for WSCP radioactivity. The filled circles represent the A_{260} values for HA after subtraction of the small contribution from WSCP.

coefficient assumes that the binding capacity of HA for these substances is unlimited. In the present investigation with WSCP, it was determined that the binding interaction with HA was saturable and, therefore, the use of a partition coefficient to describe the interaction is inappropriate. Hence the K_D and B_{max} values. The theory behind the use of partition coefficients with many substances is that these substances have limited water solubility and when they interact with HA the HA forms what can best be described as "micelle-like" structures with the substance (18). This could be characterized as similar to the way in which detergents form micelles with oil. HA which have both hydrophilic and hydrophobic domains will attract unlimited amounts of the substances with limited water solubility and more or less form a shell of HA molecules around these to protect them from interaction with the highly polar water molecules. Since WSCP is highly water soluble this model is inappropriate. Our data support this fact.

Strictly for the purposes of comparison, values equivalent to the partition coefficient have been calculated for WSCP based on the K_D and B_{max} values quoted in the results section. These values, for WSCP concentrations of 0.064 mg/L and 6.4 mg/L, are also presented in Table 2. It is interesting that they are of the same magnitude as the partition coefficients available from the literature for a variety of substances of limited water solubility. It must be kept in mind, however, that the use of partition coefficients is inappropriate with WSCP because the binding interaction with HA is saturable.

Investigators should use caution and not automatically assume that interactions of slightly soluble organic substances with HA are unsaturable. A wide range of concentrations (at least 10,000 fold) must be tested to confirm unsaturability.

Table 2. Partition coefficients for various toxic and hazardous substances with Aldrich[R] humic acid (HA)*

Compound	Log P**	Method	[HA] (mg/L)
p,p'-DDT	5.4[a]	dialysis	9.5
p,p'-DDT	5.1[a]	reverse phase	9.5
p,p'-DDT	5.6[b]	dialysis	8.5
anthracene	4.5[a]	dialysis	9.5
anthracene	4.0[a]	reverse phase	9.5
anthracene	4.4[c]	dialysis	
biphenyl	3.0[a]	reverse phase	9.5
TCB***	5.3[a]	reverse phase	9.5
TCB***	4.5[a]	dialysis	9.5
WSCP (0.064 mg/L)****	4.7	calculation	9.5
WSCP (6.4 mg/L)****	4.3	calculation	9.5

*The Aldrich[R] HA used for these data was the acid precipitable fraction.

**Log P is the log of the partition coefficient. The letters in superscript refer to the literature source for the value.

***2,5,2',5'-tetrachlorobiphenyl

****The P values for WSCP were calculated as ng WSCP bound per g HA divided by ng WSCP free per g water using the B_{max} and K_D values given under the heading summary of findings. Since the binding of WSCP to HA is saturable the value of the partition coefficient will be dependent on the WSCP concentration. These values are invalid because of this consideration and are presented only for the purpose of comparison with literature values for other compounds.

[a]Landrum et al. (19)

[b]Carter and Suffett (20)

[c]Carter (21)

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