Binding of Atrazine to Humic Substances from Soil, Peat, and Coal Related to Their Structure†

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Partition coefficients for the binding affinities of atrazine to 16 different humic materials were determined by the ultrafiltration HPLC technique. Sources included humic acids (HA), fulvic acids (FA), and combined humic and fulvic fractions (HF) from soil, peat, and coal humic acid. Each of the humic materials was characterized by elemental composition, molecular weight, and composition of main structural fragments determined by 13C solution-state NMR. The magnitude of KOC values varied from 87 to 575 L/kg of C, demonstrating relatively low binding affinity of humic substances (HS) for atrazine. On the basis of the measured KOC values, the humic materials can be arranged in the following order: coal HA = gray wooded soil HA > chernozemic soil HA and HF > sod-podzolic soil HA = peat HF > sod-podzolic soil FA ≫ peat dissolved organic matter. The magnitude of the KOC values correlated strongly with the percentage of aromatic carbon in HS samples (r = 0.91). The hydrophobic binding was hypothesized as the key interaction underlying the binding of atrazine to HS.

Introduction

Atrazine (2-chloro-4-(ethy lamino)-6-isopropylamino-triazine) is one of the most widely applied and persistent herbicide. Elevated concentrations have been found in groundwater, rivers, lakes, and soils (1). The fate of atrazine in the environment can be greatly affected by humic substances (HS) (2, 3), which comprise from 50 to 80% of natural organic matter in water and soil ecosystems. So, it is expected that the binding to HS affects degradation rate and toxicity of atrazine (2–5).

A partition coefficient (KOC) can be used as a quantitative measure of the magnitude of binding affinity of HS for atrazine. In addition, it allows the calculation of the portion of herbicide bound to HS that is of importance for prediction of the atrazine fate in the soil and water environment. As a result, several investigations have been devoted to determination of KOC for atrazine to HS (6–13). The reported KOC values differ substantially in their magnitude (from 25 to 600 L/kg of C). This could be connected to the various reactivity of the humic materials having different structure; however, the nonoptimum analytical approach used for studying the atrazine–HS interaction can contribute as well. Given the high structural heterogeneity and irregularity of HS, another important factor could be the limited number of target humic materials (not exceeding three preparations) used for the previous studies. Until the present, a common opinion is missing concerning both the binding mechanism of atrazine to HS and the structural features responsible for the binding (6, 9, 10, 14–19). Wang et al. (16) reported the preferential binding of atrazine to the higher molecular weight fraction of fulvic acids and proposed weak mechanisms of interaction, such as hydrogen bonding and hydrophobic bonding, to interpret their findings. Hydrogen bonding was also suggested as a governing mechanism by Sullivan and Felbeck (15) and Welhouse and Bleam (17), who suggested that particularly strong complexes could be expected between atrazine and acid functional groups of HS. Piccolo et al. (18) and Mueller-Wegener and co-workers (19, 20) are in favor of a charge-transfer mechanism. However, the spectroscopic studies of Martin-Neto et al. (21) on this subject have indicated that a charge-transfer mechanism was not operative in atrazine–HS interaction. They gave evidence instead for a hydrogen bonding mechanism.

In this paper, we have determined the partition coefficients for a large set of humic materials of different origin (16 samples) including HS from different sources (soil, peat, and coal) as well as fractional composition (HA vs FA). We have used a broad range of molecular descriptors, including more specific 13C NMR descriptors for deriving the structure–atrazine binding affinity relationships (22).

Our objectives were to (i) measure the KOC of atrazine for a variety of humic sources and (ii) derive the correlation relationships between the atrazine binding affinities and the molecular descriptors of HS.

Experimental Section

Atrazine (99.97%) was purchased from Dr. Ehrenstorfer GmbH (Germany). Stock solution of 10 mg/L was prepared in distilled water (pH 5.5) and stored in the dark at 4 °C.

Humic materials (16 samples) used in this study were isolated from soil and peat and also included commercial humic acid from brown coal.

Soil humic acids (HA) were isolated from eight soils. These included five sod-podzolic soils near Moscow (related to the Spodosols) sampled from a forested site (HBW and HBW1), from cultivated soil (HBP1), and from a garden (HBB, HBG1); one gray wooded soil (related to the Alfisols) near Tula (HGW); and typical and meadow chernozems (related to the Mollisols) near Voronezh (HST and HS, respectively).

Soil fulvic acids (FA) were isolated from three sod-podzolic soils above near Moscow sampled from a forested site (FBW1), from cultivated soil (FBP1), and from a garden (FBBG1). All the sampling sites are in Russia. The HA extraction was performed as described in ref 23. In brief, a soil sample was extracted with 0.1 M NaOH. For calcareous soils (chernozems), the sample was first treated with 10% HCl. The alkali extract was treated with 0.3 M KCl to remove the organomineral colloidal particles. The HA and FA were obtained by standard fractionation technique using acidification of the extract to pH 1–2. The precipitated HA were desalted by dialysis. To isolate FA, the common XAD technique was used as described elsewhere for isolation of the aquatic HS (24). In brief, the acidic supernatant was passed through XAD-2 resin. The sorbed fraction of FA was recovered with 0.1 M NaOH and desalted with the use of a cation-exchanger resin.

Soil humic substances (nonfractionated, soil HF) were isolated by alkaline extraction from typical chernozem near Stavropol (SEL).

1 This paper is dedicated to the 60th birthday of Professor Fritz Hartmann Frimmel.

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Peat humic substances (nonfractionated, peat HF) were isolated from the highland sphagnum peat (T4). Isolation procedure was as described elsewhere (25) and included preliminary treatment of a peat sample with an ethanol–benzene mixture (1:1 v/v) followed by an extraction with 0.1 M NaOH.

Water extract from woody-herbaceous peat (HTW) was prepared by adding distilled water to the peat sample (2:1, v/v). The suspension obtained was continuously stirred for 12 h. The water phase was filtered off through a 0.45-μm membrane filter followed up with desalination using a cation exchanger and concentrated using a rotor evaporator.

Peat humic acid (HTO) was a commercial preparation purchased from Biolar (Olaine, Latvia).

Coal humic acid (AGK) was a commercial preparation kindly provided by Biotechnology Ltd. (Moscow, Russia).

Structural Characterization of HS. Elemental analyses (C, H, and N) were conducted on a C, H, N analyzer (Carlo Erba Strumentazione 1106). Ash content was determined gravimetrically. The contents of all the elements were calculated on an ash-free basis. Oxygen contents were calculated as the difference between a weight of the ash-free sample analyzed and the sum of the C, H, N contents found. Moisture was not accounted for.

SEC analysis was performed at the facilities of the Division of Water Chemistry, Engler-Bunte Institute, Technical University of Karlsruhe, Germany, according to ref 26. Toyopearl HW-50S gel was used for column packing. Polydextrins were used as markers for molecular weight calculations. HS solutions were prepared at a concentration of 1–2 mg/L by equilibrating with the SEC mobile phase (0.028 M phosphate buffer, pH 6.8) prior to the analysis.

Quantitative 13C solution-state NMR spectra were recorded on a Varian VXR-400 spectrometer operating at 100 MHz. A weight of HS sample of 100 mg was dissolved in 3 mL of 0.1 M NaOD and transferred into a 10-mm NMR tube. All the spectra were recorded at 4–8 s delay time using inverse gate decoupling. These conditions were shown to provide quantitative determination of carbon distribution of the main structural fragments of HS (27). To quantify the observed spectra, the assignments were made after Kovaleski et al. (27) and were as follows (in ppm): 5–108, aliphatic non- and O-substituted C atoms (ΔCAl); 108–165, aromatic non- and O-substituted C atoms (ΔCAr); 165–187, C atoms of carboxylic and esteric groups (ΔCCO); 187–220, and C atoms of quinonic and ketonic groups (ΔCCO). To derive the molecular descriptors, the percentage of carbon in the given structural fragments was used.

Stock solutions of HS were prepared by dissolving a weight of 150 mg of air-dried sample in 10 mL of 0.1 M NaOH under continuous stirring. pH of the solution was adjusted to 5.5 using 0.1 M HCl and NaOH. Then, the solution was brought to a final volume of 50 mL using distilled water.

Binding Experiments. For measuring partition coefficients of atrazine for HS, a series of five atrazine–HS solutions were prepared. The concentration of atrazine in all the solutions was 2 mg/L, and the concentration of HS was adjusted in the range of 0.5–1.5 g of C/L by dilution to the stock solution of HS (3 g of C/L). The solutions were thoroughly mixed for 24 h at room temperature. The duration of the equilibration period was selected based on the findings reported in ref 28. All the binding experiments were conducted in three replicates.

The species of atrazine freely dissolved in water and bound to HS were separated using ultrafiltration technique. The experimental design described in ref 29 used an Amicon ultrafiltration cell of a maximum volume of 10 mL and a filter membrane YM-2 of molecular weight cutoff of 1000 Da. When not in use, the membrane was stored in distilled water at 4 °C. The prepared HS–atrazine solutions were transferred into the ultrafiltration cell and filtered with continuous stirring at 4.6 atm. The concentration of organic carbon in the ultrafilterate did not exceed 1 mg/L accounting for less than 0.1% of the initial concentration of HS used. We thus disregarded the portion of the HS-bound atrazine that leaked through the membrane.

The determination of atrazine in the ultrafiltrate was conducted using the HPLC technique as described elsewhere (16). The HPLC system used was Gold Model 126 equipped with UV detector. The UltraspHERE Beckman column 4.6 mm × 15 cm was used for separation. A mixture of acetonitrile and water (50:50, v/v) containing 3.18 × 10–4 M HCl (pH 2.5) was used as the mobile phase. The absorbance of eluate was detected at 220 nm.

Calculation of Partition Coefficients of Atrazine for HS (KOC). The binding of atrazine (A) to HS can be quantitatively described by the following equilibrium constant:

$$K = \frac{[\text{A-HS}]}{[\text{A}][\text{HS}]}$$

where [A] and [A – HS] are the concentrations of atrazine nonbound (passing through the ultrafilter) and HS-bound (retained by the filter), respectively, and [HS] is the equilibrium concentration of HS.

As the total concentration of HS (CiHS) was always much larger than that of atrazine (Ca), [HS] can be put equal to CiHS. Because of the unknown stoichiometry of the interaction of atrazine with HS, the total concentration of HS is expressed, as a rule, on the mass basis with dimensional units of kilograms of C per liter (3). In this case, eq 1 can be transformed into the following expression for partition coefficient (KOC) of atrazine to HS:

$$K_{OC} = \frac{1 - \alpha}{c_{iHS}}$$

where $\alpha = [\text{A}] / c_A$ and $1 - \alpha = [\text{A} - \text{HS}] / c_A$ are the portion of atrazine nonbound and HS-bound, respectively. Therefore, the KOC of atrazine can be determined as the slope of a linear plot, which can be described by the following experimental relationship:

$$c_A = K_{OC}c_{iHS} + 1$$

As the concentration of HS is usually expressed in kilograms of C per liter, the dimensional units of the above KOC value was liters per kilogram of C. The above equation is similar to the Stern–Volmer equation widely used for determination of KOC of polycyclic aromatic hydrocarbons (PAH) for HS (30).

Results and Discussion

Partition Coefficients. Typical plots of the relationship of $c_A / [\text{A}]$ versus $c_{iHS}$ are given in Figure 1 for the HS samples of different origin. They are calculated from the HPLC ultrafiltration data on the partitioning of atrazine between the species freely dissolved in the water and bound to HS. Apparently, fitting the experimental plots to the linear relationships yields high correlation coefficients ($r^2$ values varied from 0.95 to 0.99), which allows the obtained plots to be used for determination of the KOC values of atrazine for HS. The corresponding data for all the humic materials are summarized in Table 1.

The obtained KOC values for the humic materials of different origin lay in the range of 87–575 L/kg of C. The maximum KOC value of 575 L/kg of C was observed for coal HA (AGK) and HA of gray wooded soil (HGW), whereas the minimum value of 87 L/kg of C was obtained from the water.
The obtained results show clearly that the binding affinity for atrazine of the target humic materials varies greatly with respect to their source and fractional composition. Our next goal was to establish the structure-activity relationships for atrazine binding to HS.

**Relation between Structure and Binding Affinity of HS for Atrazine.** To relate atrazine binding to HS structure, we used the same approach described in detail in our previous paper on binding HS to PAH (22). It is based on a numerical description of the structure of HS by a combination of the molecular descriptors of elemental, structural fragments, and molecular weight composition. The atomic ratios (H/C and O/C) were used as the descriptors of elemental composition, the percentages of carbon in the main structural groups ($C_{Ar}$, $C_{CDO}$, $C_{CHv}$, and $C_{CAl}$) were used as the descriptors of structural fragments composition, and the weight-averaged molecular weight ($M_w$) was used as a descriptor of the molecular weight composition. The corresponding data are summarized in Table 2.

Among the molecular descriptors used, the strongest correlation with atrazine $K_{OC}$ values was observed for $\sum C_{Ar}$ and $\sum C_{CHv}/\sum C_{CAl}$. The corresponding correlation coefficients ($r$) were 0.91 (Figure 2) and 0.87, respectively, demonstrating the statistical significance of the obtained relationships at $P > 99\%$ ($n = 16$). These results are consistent with the reported studies (32) correlating $K_{OC}$ values with the percentage of aromatic carbon in the humic material. At the same time, there were no statistically relevant correlations observed with the amount of carboxylic ($C_{CDO}$) and carboxylic carbon ($C_{CAl}$), with atomic ratios, and with $M_w$.

For understanding the mechanism underlying the binding of atrazine to HS, of particular importance is that a similar relationship between $K_{OC}$ values and aromaticity of HS was observed for binding of PAH to HS (22). The more prevalent the hydrophobic aromatic core is as compared to the hydrophilic (mostly polysaccharide) aliphatic periphery, the greater the hydrophobicity of the HS. Thus, the obtained results could have been interpreted as a key role of hydrophobic interactions in binding of atrazine to HS as was previously reported (7, 10, 11, 16). The charge-transfer interaction might also contribute in binding of atrazine to HS as was hypothesized by the different investigators (6, 18–20). A lack of correlation of the obtained $K_{OC}$ values with the percentage of carboxylic and carboxylic carbon does not corroborate the reported findings, suggesting a dominant role of hydrogen bonding or proton transfer in atrazine binding to HS (15, 17, 21). The above mechanisms can prevail at pH < 4 when atrazine becomes protonated (28). However, such conditions are not environmentally relevant, which makes the mechanisms of ion exchange and proton transfer hardly feasible as an explanation of the binding of atrazine to HS in soil and water ecosystems.

**Environmental Implication.** The $K_{OC}$ values reported in this paper are of major importance for prediction of atrazine behavior in different aquatic and soil media. As indicated by the $K_{OC}$ values obtained, the toxicity of atrazine applied to agricultural crops will depend not only on the content of humic matter in the soil but also on its quality as well. Thus, the same application rate of atrazine is expected to result in the highest herbicidal impact on the mollisol (chernozem) containing the largest amount of HS of highly aromatic character and result in the greatest herbicidal impact on the humic depleted, tilled sod-podzolic soils that contain HS of predominantly aliphatic character. Our experimental estimates of the toxicity of atrazine introduced into soils of

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**TABLE 1. Partition Coefficients of Atrazine ($K_{OC}$) for Humic Substances Used in This Study**

<table>
<thead>
<tr>
<th>sample</th>
<th>source of HS</th>
<th>$K_{OC}$b</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4</td>
<td>sphagnum peat</td>
<td>377 ± 23</td>
</tr>
<tr>
<td>HTW</td>
<td>water extract of peat</td>
<td>87 ± 5</td>
</tr>
<tr>
<td></td>
<td><strong>Soil Humic Acids</strong></td>
<td></td>
</tr>
<tr>
<td>HBW</td>
<td>sod-podzolic soil, forest</td>
<td>380 ± 20</td>
</tr>
<tr>
<td>HBG</td>
<td>sod-podzolic soil, garden</td>
<td>400 ± 24</td>
</tr>
<tr>
<td>HBW1</td>
<td>sod-podzolic soil, forest</td>
<td>281 ± 17</td>
</tr>
<tr>
<td>HBP1</td>
<td>sod-podzolic soil, tillage</td>
<td>181 ± 30</td>
</tr>
<tr>
<td>HBG1</td>
<td>sod-podzolic soil, garden</td>
<td>380 ± 23</td>
</tr>
<tr>
<td>HGW</td>
<td>gray wooded soil, forest</td>
<td>575 ± 34</td>
</tr>
<tr>
<td>HS</td>
<td>meadow chernozem soil</td>
<td>501 ± 31</td>
</tr>
<tr>
<td>HST</td>
<td>typical chernozem soil</td>
<td>404 ± 23</td>
</tr>
<tr>
<td></td>
<td><strong>Soil Fulvic Acids</strong></td>
<td></td>
</tr>
<tr>
<td>FBW1</td>
<td>sod-podzolic soil, forest</td>
<td>192 ± 12</td>
</tr>
<tr>
<td>FBP1</td>
<td>sod-podzolic soil, tillage</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>FBG1</td>
<td>sod-podzolic soil, garden</td>
<td>275 ± 17</td>
</tr>
<tr>
<td>SEL</td>
<td>typical chernozem</td>
<td>444 ± 25</td>
</tr>
<tr>
<td></td>
<td><strong>Commercial Preparations</strong></td>
<td></td>
</tr>
<tr>
<td>HTO</td>
<td>peat humic acid</td>
<td>300 ± 20</td>
</tr>
<tr>
<td>AGK</td>
<td>coal humic acid</td>
<td>575 ± 35</td>
</tr>
</tbody>
</table>

* Values correspond to a confidence interval at $n = 3$ and $P = 95\%$. 

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extract from peat (HTW). The obtained values agree well with the reported $K_{OC}$ values for atrazine binding to dissolved humic materials determined by different analytical techniques ranging from 41 to 600 L/kg of C (8, 9, 11–13). According to the obtained data, the maximum binding capacity of HS for atrazine can be estimated to be as large as 0.9 mg of atrazine/g of C or 12 µmol of atrazine/g of HS, which is in the range of the previously reported values varying from 2.2 (16) to 21.3 (31) µmol of atrazine/g of HS. A comparison of the atrazine $K_{OC}$ values with those for PAH obtained for the same humic materials (22) shows that the atrazine $K_{OC}$ are 2–3 orders of magnitude lower than those for PAH.

On the basis of the measured $K_{OC}$ values, the target humic materials can be arranged in the following order of affinity for atrazine: coal HA > gray wood soil HA > chernozemic soil HA and HF > sod-podzolic soil HA > peat HF > sod-podzolic soil FA > peat DOM. The soil HA samples studied had the highest binding affinity for atrazine than FA extracted from the same soil. It is of importance to note that the binding affinity of both HA and FA extracted from the garden sod-podzolic soil was higher than that of the HA and FA extracted from the virgin (a forested site) and cultivated sod-podzolic soils. Among the soil humic materials studied, the HA and FA isolated from the cultivated sod-podzolic soil had the lowest binding affinity for atrazine but was much higher than that of the water extract of peat.
different type and use showed that, as expected from the content and aromaticity of HA in soils, the lowest toxicity of atrazine at the same application rate was observed for mollisols (typical and meadow chernozem) (33).

On the other hand, the structure–activity relationships for atrazine binding to HS point out that aromatic-rich humic materials are the most efficient binding agents for atrazine. Hence, the coal humates can be effectively used to minimize the residual toxicity of the atrazine-treated agricultural fields. The advantage of humate application is their combined action as detoxifying agents and organic fertilizers. The quantification of detoxifying properties of humic materials in relation to atrazine and other herbicides will facilitate an effective use of humates for detoxification of bound residues of herbicides.

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