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Soil Leaching Column Chromatographic Technique for Estimation of Leaching Behavior of Atrazine, Deethylatrazine, Deisopropylatrazine, and Hydroxyatrazine on Soil

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Agriculture production has greatly benefited from the use of herbicides. Nevertheless, their extensive use can contaminate groundwaters by their leaching through soil porosity. *S*-triazines are the sort of herbicides that have been frequently detected in surface and ground waters primarily because of their widespread use and moderate persistence in soil (Poinke et al. 1988). Atrazine (AT; 2-chloro-4-ethylamino-6-isopropylamino- *s*-triazine) is applied to control the growth of grasses mainly in corn and sorghum (Mersie and Seybold 1996). Field-monitoring studies of surface and ground waters have shown the detection of AT and its three metabolites deethylatrazine (DEA; 2-amino-4-chloro-6-isopropylamino- *s*-triazine), deisopropylatrazine (DIA; 2-amino-4-chloro-6-ethylamino- *s*-triazine), and hydroxyatrazine (HA; 2-hydroxy-4-ethylamino-6-isopropylamino- *s*-triazine) (Thurman et al. 1994; Adams and Thurman 1991; Poinke et al. 1988). Besides AT, its metabolites are also phytotoxic and toxic for animals (Behki and Khan 1986), they are formed by microbial degradation through N-dealkylation (Poinke and Glotfelty 1990) or hydrolysis of AT (Mandelbaum et al. 1993). Sorption behaviors of AT and its metabolites with batch equilibrium method were extensively studied, but few studies have focused on their retention by soils. Since available data were not always obtained under the same conditions for the four compounds (Brouwer et al. 1990), a direct comparison of their leaching ability is almost impossible.

As detailed screening of herbicide's mobility behavior under field conditions is time-consuming and costs a lot, reversed-phase (RP) HPLC has been proposed (Singh et al. 1990). Hansch et al. (1979) demonstrated the relationship between logarithm of capacity factor (k') on an RP-HPLC column and logarithm of octanol/water partition coefficient (log K_{ow}) of a chemical. A linear correlation also exists between log Kow and log Koc (Sabljic et al. 1995), the soil organic carbon distribution coefficient. Thus a correlation between measured log K_{oc} and log k' in RP-HPLC is present (Kördel et al. 1993). However, in most cases, these correlations are only suitable for the solutes that have similar interactions with two chromatographic phases, or for the same class of compounds. Otherwise large estimation error will occur when all solutes with different classes are treated as a single group. The objective of our study was to use a new soil

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leaching column chromatographic technique (Xu et al. 1999) to estimate and compare the leaching potential of AT and its three metabolites in a soil. The work was done in the laboratory with a reference soil as a stationary phase and water as an eluent. The *k'* values of the four herbicides in the chromatography were measured, and were contrasted with mobility data of literatures. The correlations between log *k'* and log *K*oc for the four herbicides on soil column and octadecylsilane (C_{18}) column were also compared.

MATERIALS AND METHODS

Reference compounds of AT, HA, DEA, and DIA were purchased from Aldrich (Steinheim, Germany) and Fluka (Buchs, Switzerland), whose purities were >98%. Water was generated by an Elix 5 + Milli-Q gradient purifying system (Millipore Corporation, Cedex, France). All other used chemicals are analytical or HPLC grade. A reference soil (GSE 17201) was obtained from Bayer Landwirtschaftszentrum, Moheim, Germany and passed through a 2-mm sieve. The main physicochemical characteristics of the soil were as follows: pH in 0.01M $CaCl_2$ 6.3, organic carbon content 2.48%, cationic exchange capacity 10 meq 100 g^{-1}, sand 80.5%, silt 12.3%, and clay 7.2%. 14-g soil was divided into eleven portions, and each portion was dry-packed into a stainless steel column (10 mmi.d. ×100 mm) with the same and constant packing height by a homemade pressurizing device. Both column ends contained organic membranes (pore size, 0.45 pm) and stainless steel flow-rate allocation discs. The bulk density was 1.9 g mL^{-1}.

The retention of each compound was determined by a HPLC system. The system consisted of two Waters 510 pumps, a Rheodyne 7725i injection value, a Hypersil C_{18} column (5 pm, 4.6 mmi.d.× 250 mm) thermostated at 25.0±0.1 °C, a UV 486 detector set at the λ_{max} of each compound, and a Waters 800 PC integrator. The binary mobile phase consisted of different volume ratio of methanol/water: 90:10, 80:20, 70:30, 60:40, and 50:50 at isocratic elution mode. The flow rate was 1.0 mL min^{-1}. The dead volume of the system was measured by injecting a 1% $NaNO_2$ solution at 220 nm. Retention time was measured by injecting 10 µL of a compound in a methanol solution at a concentration ranging from 10 to 50 µg mL^{-1}, depending on the λ_{max} of the compound. The injection was made in duplicate. The capacity factor was calculated according to $k'=(t_r - t_0)/t_0$, where t_r and t_0 were the retention times of the solute and of $NaNO_2$, respectively.

The leaching measurement was conducted in the same system, except that the soil column was as a leaching column and pure water as a mobile phase. It took about 1 h to precondition the new soil column with pure water at 1 mL min^{-1}, then a flat baseline was observed and a stable pump pressure of about 5 MPa was maintained. Afterwards, the capacity factor (*k'*) of each herbicide could be determined as above mentioned in HPLC. The retention time of $NaNO_2$ is shortest among the chemicals having been tested, so the dead time (t_0) of the system was also measured with $NaNO_2$ solution.

RESULTS AND DISCUSSION

On C_{18} column, the stronger the molecular polarity of a solute is, the shorter the retention time is. When Cl atom of AT is replaced by an OH radical to form HA, the capacity factor (k') will get smaller due to increasing solute's polarity. The *k'* values of DEA and DIA are smaller than that of AT due to dealkylation of alkylamino radical to become relative stronger polar amino radical. AT lose an ethyl to form DEA, or lose an isopropyl to form DIA, the *k'* value of DIA is smaller than that of DEA because of a slightly stronger polarity of DIA than DEA. The experimental *k'* data on Hypersil C_{18} (Table 1) were the case of increasing in the order of DIA<DEA<HA<AT. The retention equations of log *k* of the herbicides *vs* volume fraction of methanol (φ) in MeOH-H_2O eluent are also shown in Table 1. With different φ data, the same *k'* sequent order was observed.

Table 1. Capacity factors (*k'*) of *s*-triazines on Hypersil C_{18} and soil leaching column*

No.	Solutes	*k'* on C_{18}						k'_w in SLC
		φ=0.9	φ=0.8	φ=0.7	φ=0.6	φ=0.5	Retention equation	
1	HA	0.589	0.809	1.290	2.337	5.295	$\log k'=1.826-2.355\varphi$ (r=0.984)	21.18
2	AT	0.688	1.006	1.823	3.492	7.810	$\log k'=2.162-2.650\varphi$ (r=0.992)	3.537
3	DIA	0.459	0.537	0.680	0.869	1.213	$\log k'=0.586-1.052\varphi$ (r=0.992)	2.089
4	DEA	0.500	0.620	0.863	1.243	2.038	$\log k'=1.032-1.523\varphi$ (r=0.990)	1.728

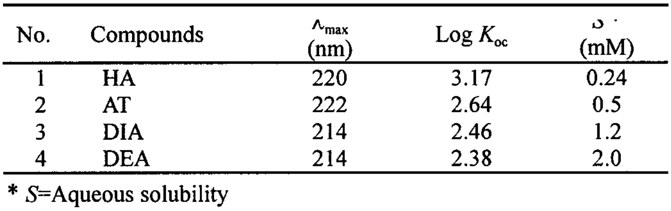
* φ= Methanol volume fraction in methanol-water eluent; k'_w= *k'* value with pure water as eluent; SLC=Soil leaching column.

K_{oc} is an important parameter incorporating into mathematical models for predicting the environmental fate of a pesticide (July et al. 1983). It is generally measured from $K_{oc}= (K_d/OC\%)\times 100$, where OC% is the organic C content of soil and K_d is a partition coefficient of a compound between the concentration bound to the soil (μg kg^{-1}) and the concentration in solution (μg mL^{-1}) after equilibrium (Mersie and Seybold 1996). Not only is it very laborious to determine K_{oc}, but also the measured K_{oc} is very dependent on the properties of used soil. Here the log K_{oc} values of the four herbicides determined by Mersie and Seybold (1996) with a kind of soil are presented in Table 2, accompanied with their aqueous solubilities (Erickson and Lee 1989).

Shea (1989) suggested that K_{oc} values were negatively correlated with aqueous solubilities for the same class of compounds. In our work, only a relatively rough relation was observed, $\log S= -1.08 \log K_{oc}+2.73$ (r=0.940). The increasing order of sorption of the *s*-triazines onto the soil roughly follows the order of decreasing aqueous solubility, which is DEA, DIA, AT and HA. Additionally, the K_{oc}, values followed the order DEA<DIA<AT<HA, suggesting that the affinity for the organic matter bound to the soil

increased in the order DEA<DIA<AT<HA, which is entirely different from the k' order on C_{18} column. The types of bonding involved in *s*-triazines sorption by soil organic matter include van der Waals forces, hydrogen bonds, charge transfers, ionic bonds etc. The hydrogen bonding is of particular importance for HA compared to AT, DEA and DIA, and thus HA has a great adsorption and retention. Besides, maxium adsorption occurs at pK_a values of *s*-triazines (Weber et al. 1969). The pK_a values for HA, AT, DIA and DEA are 5.2, 1.7, 1.5 and 1.4, respectively (Seybold and Mersie 1996). HA has a pK_a that is near to the pH of the soil, which indicates a higher degree of adsorption than that for AT, DEA and DIA. Its relatively large pK_a coupled with its low water solubility helps explain the relatively high degree of adsorption observed for HA onto the soil. Lerch et al. (1997) reported that mixed-mode sorption, involving both cation exchange and hydrophobic interactions, was the mechanism determined for stronger binding of HA.

Table 2. Summary of physicochemical parameters of *s*-triazines



No.	Compounds	λ_{max} (nm)	Log K_{oc}	S^* (mM)
1	HA	220	3.17	0.24
2	AT	222	2.64	0.5
3	DIA	214	2.46	1.2
4	DEA	214	2.38	2.0

* *S*=Aqueous solubility

Although the four *s*-triazines are in the same class, the correlation coefficients (*r*) of log *k'* on C_{18} column *vs* log K_{oc} are only from 0.51 to 0.61 for different isocratic methanol fraction (φ) from 0.5 to 0.9. The reason may be that the solutes have different interaction mode on C_{18} from that on soil, and the solute with larger K_{oc} value could not mean that it had a longer retention time on C_{18} column. So even though for the same class of compounds (like *s*-triazines), it is also very dangerous to predict a compound's K_{oc} value from its *k'* on C_{18} column.

Nevertheless on soil column, a similar soil-water environment exists for determination of either K_{oc} or *k'*. So there should be a better correlation between log K_{oc} and log k'_w, the log *k'* value but with soil as a stationary phase and water as a mobile phase. Table 1 lists k'_w values, and Figure 1 shows the significant correlation: $\log k'_w = 1.395 \log K_{oc} - 3.107$, with *r* value of 0.999. HA had the largest k'_w and K_{oc}, and was most strongly adsorbed by the soil. AT had intermediate k'_w and K_{oc}, DIA and DEA had smaller k'_w and K_{oc} values and were weakly adsorbed. Therefore, the soil leaching column chromatographic technique could provide a potential tool for rapid prediction of a compound's K_{oc} through its measured k'_w value.

The k'_w order of herbicides also enabled us to draw conclusions concerning the comparative risks of groundwater contamination by leaching of AT, HA, DEA, and DIA

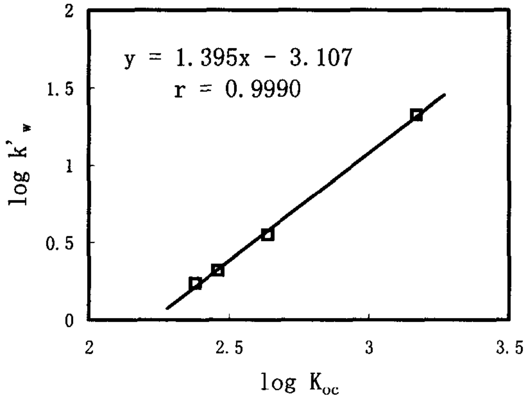


Figure 1. Correlation of log k'_w from soil leaching column chromatography with log K_{oc} for four *s*-triazines

through a natural soil system. A possible contamination by HA would occur much later, due to the largest k'_w, than that by AT, DIA, and DEA. On the contrary, DEA and DIA have a greater tendency to leach than AT. This helps explain the higher concentration of DIA and DEA than AT in groundwater (Pionke and Glotfelty 1990), and DEA to be less adsorbed than AT in soils (Brouwer et al. 1990; Adams and Trurman 1991). HA is more strongly adsorbed in soils than AT, and has a high affinity for organic matter bound to soils and a much lower tendency to leach (Brouwer et al. 1990; Raju et al. 1993). These results were all consistent with our k'_w order. In the present work, the decreasing of compound's concentration due to degradation on soil column may not be considered, the reasons are as follows: firstly, *k'* determination is quick, in contrast to degradation half-life of herbicides; secondly, even though some herbicides may decrease their concentrations owing to degradation during that time, from the point of view of linear chromatography, the *k'* is mainly influenced by solute itself, not by its concentration; thirdly, it is easy to know whether a new metabolite is generated during experimental course through recorded chromatogram. In our chromatograms, there was no new peak appearing.

For the chemical that has even larger k'_w value than the studied *s*-triazines, mixture of methanol-water instead of pure water could be utilized as an eluent to shorten retention time. A significant linear correlation between log *k'* with mixed eluent and log K_{oc} was also observed (Xu et al. 1998), which enabled us more rapidly to get valuable knowledge of the likely behavior of chemicals and hence the resulting risk for groundwater contamination.

In summary, mobility of a chemical through soil is a primary factor in assessment of its environmental fate. It is useful in the preliminary evaluation of new or existing chemicals to have a rapid means to estimate mobility potential through laboratory measurements. Soil leaching column chromatography is superior to RP-HPLC in providing this type of tool for estimation of K_{oc} values and tendency of chemicals to leach through soil.

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