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Octanol/Water Partition Coefficient of Selected Herbicides: Determination Using Shake-Flask Method and Reversed-Phase High-Performance Liquid Chromatography

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For some triazines and phenylurea derivatives no reliable K_{OW} values were available from literature. The log K_{OW} values were determined directly with the shake-flask procedure and also indirectly using the reversed-phase high-performance liquid chromatography (RP-HPLC) method in the isocratic and gradient elution mode, respectively. Atrazine, as a well-investigated substance, and two herbicides of different chemical structures (acid amides) were included in this study. Both RP-HPLC methods yield log K_{OW} values comparable among themselves and identical with the shake-flask results for methabenzthiazuron (2.5), chloroxuron (3.8), sebutylazine (3.2), and atrazine (2.6). For the atrazine metabolites investigated, the RP-HPLC-derived log K_{OW} values are significantly less than those directly measured (1.0 for desethylatrazine and 1.5 for desisopropylatrazine). In contrast to these findings, considerably higher log K_{OW} values were obtained for the two acid amides propachlor and metazachlor with both RP-HPLC methods compared to the shake-flask values (2.2 for both compounds). Because of the still complicated choice of reliable reference compounds/data for the RP-HPLC methods, the shake-flask technique should be preferred for determination of octanol/water partition coefficients with high accuracy in the range of log K_{OW} < 4. Finally, the directly measured K_{OW} values were compared with estimates obtained with two widely applied group-contribution methods (CLOGP and LOGKOW).

Introduction

Triazines and phenylurea derivatives are widely used as plant-protecting agents in modern agriculture. They have moderate aqueous solubilities and were found as micropollutants in the aquatic environment, for example, in the Elbe River drainage system in Germany[1,2] or in the Rivers Seine and Marne in France.[3] The octanol/water partition coefficient (K_{OW}) is an important physicochemical descriptor for assessing transport pathways and environmental partitioning (e.g., sorption or bioconcentration) of such herbicides.[4,5] Current K_{OW} data compilations[6−10] show considerable gaps or discrepancies among the cited K_{OW} values for a number of herbicides. Especially for the triazines desethyl- and desisopropylatrazine (metabolites of atrazine) and sebutylazine and for the phenylurea derivative methabenzthiazuron we found no reliable data in the literature. For a further phenylurea of practical relevance, chloroxuron, the recommended log K_{OW} value[7] seems to be an outlier from the series, and therefore this compound was included in the actual study.

The K_{OW} values of the selected herbicides were measured, on the one hand, directly using the shake-flask procedure[11] and for comparison also with the indirect reversed-phase high-performance liquid chromatography (RP-HPLC) method. The last-mentioned technique is used in two ways, first according to a standard protocol[12] with isocratic elution and second in a more rational mode[13−15] with a linear gradient of eluting solvents. Because the evaluation of RP-HPLC measurements is sensitive to the choice of K_{OW} reference substances (which are needed for relating the retention behavior on a C_{18} column to the lipophilicity scale), only very similar compounds (triazines and phenylurea derivatives) were used for calibration. In the group of the above-mentioned test substances atrazine was included as a well-investigated example for validating our experiments, and in addition also two acid amides, propachlor and metazachlor, were included to check the applicability of the established calibration of RP-HPLC methods with substances other than the classes used as reference.

Finally, the predictive power of two widely applied group-contribution methods (CLOGP[16] and LOGKOW[17]) for log K_{OW} estimation from fragments of molecular structure was tested.

Experimental Section

Chemicals and Standards. The triazines (except the atrazine metabolites) were purchased from Supelco (Steinheim, Germany), the other herbicides under investigation from Dr. Ehrenstorfer (Augsburg, Germany), and the HPLC-grade solvents with purity >99.8% (acetonitrile and water) from Mallinckrodt-Baker (Gross-Gerau, Germany) or Merck (Darmstadt, Germany). For the shake-flask procedure 1-octanol from Merck (extrapure, purity > 99.0%) and freshly bi-distilled tap water were used. The standard solutions for quantitative HPLC analysis of samples taken during these experiments were prepared volumetrically from stocks of the mutually saturated solvents water and octanol, respectively.

Equipment and Procedure. The retention times, t_R, of the herbicides on a Spherisorb ODS 2 (5 μm) stationary

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phase (from Phase Separations, Norwalk, CT) were determined substance-specifically at the wavelength with maximum absorbance (218, 230, 245, or 270 nm) using a Hewlett-Packard 1090 series II LC system with an integrated column heater and a 1040 series diode array detector. The stationary phase was filled in a refined steel column (125 mm × 4 mm) and a guard column (4 mm × 4 mm). Isocratic elution was performed with water/acetonitrile at a volume ratio of 3:1 as mobile phase. The linear gradient elution started with a mobile phase of water/acetonitrile 90:10 and decreased within 55 min to a ratio of 55:45. The water used was buffered with 1 mM ammonium acetate (pH 6.7−7.3), and both solvents were degassed by a low flow of helium (99.999% pure; Messer-Griesheim, Frankfurt a. M., Germany). The mobile phase flow rate was 0.8 mL/min in both eluting regimes, and the column temperature was constant at 40 °C. The substance peaks were identified by injecting solutions of pure compounds in acetonitrile. The holdup time of the system, t_M, was determined with sodium nitrate to be 1.52 min.

The shake-flask experiments[11] were carried out at 25 ± 2 °C, always in duplicate for each concentration level of the individual test substance. In a 50 mL test tube 15 mL of water was overlaid by 5 mL of water-saturated octanol, which contains the chemical to be equilibrated (in concentrations of 0.15−0.46 mg/mL or 0.06−0.18 mg/mL). After closing, the tube was shaken by hand for ~10 min. Then the phases were allowed to separate for ~1 h, and afterward samples of both phases were taken (the octanol phase had to be centrifuged before sampling). The octanol-phase samples were diluted with acetonitrile before analysis; the aqueous-phase samples could be analyzed directly. The quantification was done using a Merck-Hitachi HPLC system (pump L-7100; diode array detector L-4500; column thermostat) equipped with a Merck LiChroCART 250-4 column filled with Merck LiChrosphere 100 RP-18 (5 μm) as stationary phase under isocratic conditions at 25 °C with a mobile phase consisting of water/acetonitrile 7:3 (v/v) in a flow rate of 0.8 mL/min. The chromatograms were integrated at a wavelength of 220 nm.

Evaluation of Experimental Data. The retention factors under isocratic and gradient conditions, k'_{iso} and k'_{grad}, were calculated according to the equation $k' = (t_R - t_M)/t_M$. (The additional determination and consideration of the delay time of the gradient-forming system, as done by Krass et al.,[15] is not necessary because this results in only a small constant subtrahend to k'_{grad}.) The calibration lines for the RP-HPLC retention factors ($y = b_0 + b_1 \times \log K_{\mathrm{OW}}$, with $y = \log k'_{\mathrm{iso}}$ or $y = k'_{\mathrm{grad}}$) were established by linear regression with the reference data given below (Table 1). [Note that k'_{grad} can, in contrast to k'_{iso}, be evaluated directly (not log-scaled) as it was theoretically derived and experimentally verified.[15]] The 95% prediction interval of calibration lines served then for calculating confidence limits[18] for the log K_{OW} estimates from RP-HPLC retention factors.

To quantify the shake-flask samples, we have established for every test substance a five-point linear calibration line between compound peak areas in the chromatogram and its concentrations in the standard solution for the octanol and for the aqueous phase (separately). Again, the 95% prediction interval of the calibration line was used to calculate the standard deviation of the concentration.[18] This enables us (after checking the F statistics) to calculate weighted mean values of the repetitions by using the reciprocal variances as weighting factors.[19]

Table 1. Determined RP-HPLC Retention Factors log k'_{iso} and k'_{grad} for the Investigated Substances Completed by the CAS Registry No. and Recommended log K_{OW} Value (If Available)

no.	substance	CAS Registry No.	log K_{OW}[a]	log k'_{iso}[b]	k'_{grad}[c]
	Reference Substances				
1	fenuron	101-42-8	0.98	0.027	6.01
2	metoxuron	19937-59-8	1.64	0.425	12.6
3	chlortoluron	15545-48-9	2.41	0.886	20.0
4	fluometuron	2164-17-2	2.42	0.940	20.5
5	monolinuron	1746-81-2	2.30	0.965	21.0
6	diuron	330-54-1	2.68	1.028	22.5
7	isoproturon	34123-59-6	2.87	1.043	22.6
8	prometon	1610-18-0	2.97	1.074	23.0
9	linuron	330-55-2	3.20	1.431	29.3
10	hexazinon	51235-04-2	1.85	0.479	13.9
11	simazine	122-34-9	2.18	0.557	14.0
12	cyanazine	21725-46-2	2.22	0.615	15.3
13	metribuzine	21087-64-9	1.70	0.631	14.1
14	propazine	139-40-2	2.93	1.267	26.8
15	terbutylazine	5915-41-3	3.06	1.381	28.9
	Test Substances				
16	methabenzthiazuron	18691-97-9		0.870	19.6
17	chloroxuron	1982-47-4	3.20	1.900	32.3
18	desisopropylatrazine	1007-28-9		−0.353	3.25
19	desethylatrazine	6190-65-4		0.060	6.77
20	sebutylazine	7286-69-3		1.237	26.3
21	atrazine	1912-24-9	2.61	0.916	20.4
22	propachlor	1918-16-7	2.18	1.158	24.5
23	metazachlor	67129-08-2		1.187	25.0

[a] Recommended log K_{OW} taken from ref 7. [b] Average of 7 runs (relative standard deviation < 0.1%). [c] Average of 20 runs (relative standard deviation < 0.2%).

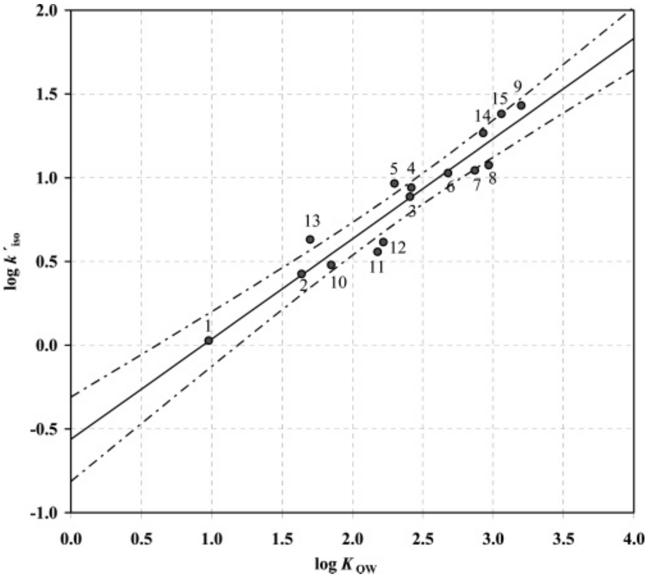

Figure 1. Calibration of the isocratic RP-HPLC method (log $k'_{\mathrm{iso}} = -0.562 + 0.598 \log K_{\mathrm{OW}}$; correlation coefficient $r = 0.9577$; residual standard deviation $s_R = 0.1162$; dotted curves indicate the 95% prediction interval of the regression line; for numeration of points see Table 1).

Results and Discussion

The RP-HPLC retention factors obtained for all substances under investigation with isocratic and gradient elution are summarized in Table 1. The calibration line for log k'_{iso} is shown in Figure 1. The data points scatter unsystematically around the straight line, that is, independent of whether the reference substance is a triazine or phenylurea derivative, and thus the inverse of the calibration line can be used for estimating the log K_{OW} of test substances from measured log k'_{iso}. Figure 2 shows the

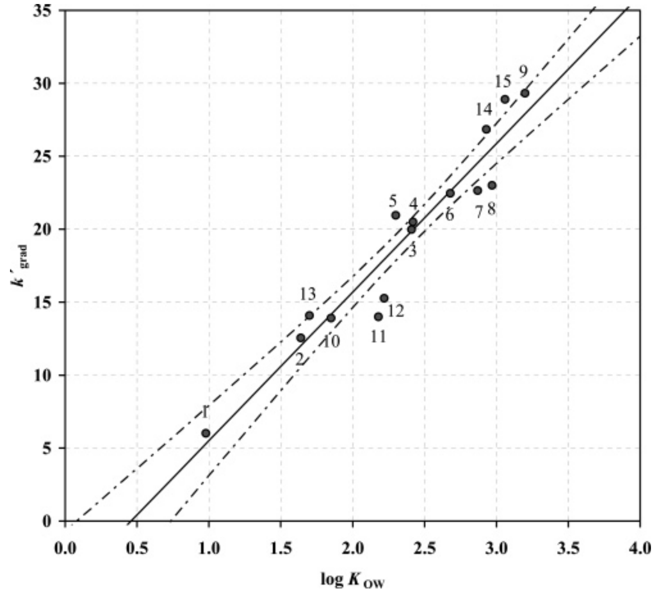

Figure 2. Calibration of the gradient RP-HPLC method ($k'_{grad} = -4.671 + 10.181 \log K_{OW}$; $r = 0.9577$; $s_R = 0.1162$; dotted curves indicate the 95% prediction interval; for numeration of points see Table 1).

calibration of k'_{grad}. Again, we have a correctly established calibration line with a random pattern of the residuals (with respect to both compound classes).

The K_{OW} values resulting for the target compounds from the measured retention factors by inversion of the shown RP-HPLC calibration lines are summarized in Table 2 together with those from the shake-flask experiments and reported experimental K_{OW} data. It can be seen that both RP-HPLC methods yield results comparable among themselves, which are for metabenzthiazuron, chloroxuron, sebutylazine, and atrazine (statistically) identical with the shake-flask results and also with reported experimental data. For the investigated atrazine metabolites the RP-HPLC-derived log K_{OW} values are less than those directly measured. The largest discrepancies were found with isocratic elution mode. In contrast to these findings, considerably higher log K_{OW} estimates were obtained for the additionally investigated acid amides propachlor and methazachlor with both RP-HPLC methods compared to the shake-flask values. This shows how sensitive the indirect RP-HPLC methods are to the choice of reference compounds. The recommended use of "structurally related substances"[11] is often not fulfilled because of a lack of reliable K_{OW} data. The still limited knowledge of the mechanism of retention in RP-HPLC and of the relevant solute- and phase-specific properties[20,21] is another factor that can hamper the appropriate choice of reference substances. It would be worthwhile to make further RP-HPLC studies with substances having a different retention behaviors like the atrazine metabolites and acid amides investigated here. Meanwhile, the authors recommend the use of the shake-flask technique for the determination of octanol/water partition coefficients with high accuracy in the range of log $K_{OW} < 4$. The modified version carried out in this study has the potential for partial automation to realize higher throughput.

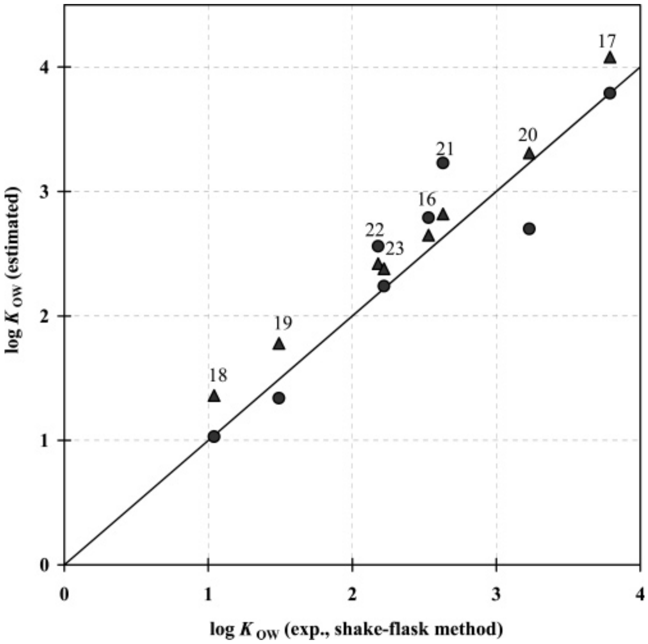

Figure 3. Directly measured versus estimated log K_{OW} values for the target compounds (numbers refer to every pair of vertical symbols: ●, CLOGP; ▲, LOGKOW; for numeration see Table 2).

In Figure 3 the log K_{OW} estimates obtained with two fragment-contribution methods are plotted against the values directly measured. As can be seen, the CLOGP estimates[16] scatter around the diagonal, whereas LOGKOW[17] overestimates slightly but systematically the experimental data. These deviations can be caused by the deficient log K_{OW} database during fragment generation. It is, however, obvious that both methods describe the trend correctly and with a precision that is, at least in some cases, superior to that of the RP-HPLC results.

Table 2. Log K_{OW} of Test Substances (with 95% Confidence Limits) Measured with One Direct and Two Indirect Methods Compared to Reported Experimentally Determined Values

		log K_{OW}			
no.	substance	shake flask	isocratic RP-HPLC	gradient RP-HPLC	lit.
16	methabenzthiazuron	2.53 ± 0.02	2.39 ± 0.15	2.38 ± 0.09	2.64[a]
17	chloroxuron	3.79 ± 0.12	4.12 ± 0.33	3.63 ± 0.22	3.70[b]
18	desisopropylatrazine	1.04 ± 0.04	0.35 ± 0.37	0.78 ± 0.27	1.15;[c] 1.01;[d] 0.78[e]
19	desethylatrazine	1.49 ± 0.01	1.04 ± 0.27	1.12 ± 0.22	1.51;[c] 1.39;[d] 1.30[e]
20	sebutylazine	3.23 ± 0.06	3.01 ± 0.19	3.04 ± 0.14	2.10;[d] 2.61[e]
21	atrazine	2.63 ± 0.07	2.47 ± 0.15	2.46 ± 0.09	2.61;[c] 2.46;[d] 2.28;[e] 2.86;[f] 2.64;[g] 2.42[h]
22	propachlor	2.18[i]	2.88 ± 0.17	2.86 ± 0.12	2.18;[i] 1.62−2.30[j]
23	metazachlor	2.22 ± 0.03	2.92 ± 0.18	2.92 ± 0.13	2.13[j]

[a] Taken from ref 17 (experimental method not specified). [b] Taken from ref 22 (modified shake-flask method). [c] Taken from ref 23 (RP-HPLC with Merck RP-18 column and methanol/water = 7:3). [d] Taken from ref 24 [RP-HPLC with octanol-coated LiChrosphere 100 (Merck) column and water]. [e] Taken from ref 24 [RP-HPLC with LiChrosphere 100 (Merck) column and a nonlinear water−methanol gradient]. [f] Taken from ref 25 [RP-HPLC with C18 (Techsphere) column and acetonitrile/water]. [g] Taken from ref 26 (shake-flask method). [h] Taken from ref 10 [RP-HPLC with Spherisorb ODS-2 (HP) column and methanol/water = 7:3]. [i] Gould, G.; Hansch, C. Pomona College, Claremont, CA, cited after ref 7 (shake-flask method). [j] Taken from ref 27 (method not specified).

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