



PII: S0045-6535(98)00368-3

DETERMINATION AND PREDICTION OF PARTITION COEFFICIENT AND TOXICITY FOR SULFONYLUREA HERBICIDES AND THEIR DEGRADATION PRODUCTS*

Liping Wei Hongxia Yu Jiasheng Cao Yue Sun Jianfang Fen Liansheng Wang**

(Pollution Control and Resources Reuse National Key Laboratory, Department of Environmental Science and Engineering, Nanjing University, Nanjing 210093)

(Received in USA 24 April 1998; accepted 13 May 1998)

ABSTRACT

The n-octanol/water partitioning coefficients ($\log K_{ow}$) of three sulfonylurea herbicides, metsulfuron methyl, chlorsulfuron and bensulfuron methyl, and five of the degradation products were determined by the shake-flask method. The capacity factors ($\log K'$) were determined by RP-HPLC with ODS column and methanol-water eluent. Correlations between $\log K_{ow}$ and $\log K'$ and molecular connectivity index were studied. Acute toxicity to the bacterium *Photobacterium phosphoreum* (15-min- $\log EC_{50}$) and the green alga *Chlorella pyrenoidosa* (96-h- $\log EC_{50}$) were determined and correlated with $\log K_{ow}$ and $\log K'$. The quantitative structure-activity-relationship equations showed that $\log K_{ow}$ and $\log K'$ are linearly correlated with toxicity, and both $\log K_{ow}$ and $\log K'$ can be used in the prediction. No significant acute toxicity of the tested compounds to the cladocera *Daphnia magna* was observed in the laboratory tests, and this was explained by ionic forms of the compounds in neutral aqueous solution.

INTRODUCTION

With the development of agriculture, herbicides are massively produced and widely applied. However there has been only limited investigation of their environmental behavior and physicochemical properties. Metsulfuron methyl, chlorsulfuron and bensulfuron methyl are widely used in agriculture in china. Saddle Hammand and Berhand M. Berger have studied their hydrolysis^[1,2], and J. Sabadie have investigated their degradation on soil^[3]. It shows that sulfonylureas are easily degraded into substituted sulfamine and heterocyclic compounds in environment, with half lives varying from days to months. Therefore, environmental behaviors, such as soil adsorption and acute toxicity, of both the sulfonylureas and their degradation products, were of serious concern.

Hydrophobicity of chemicals is an important physicochemical property, many parameters of environmental significance, such as water solubility (S_w), the adsorption coefficient for soil (K_{oc}) and bioconcentration

*This project is funded by the National Natural Science Foundation of P. R. China

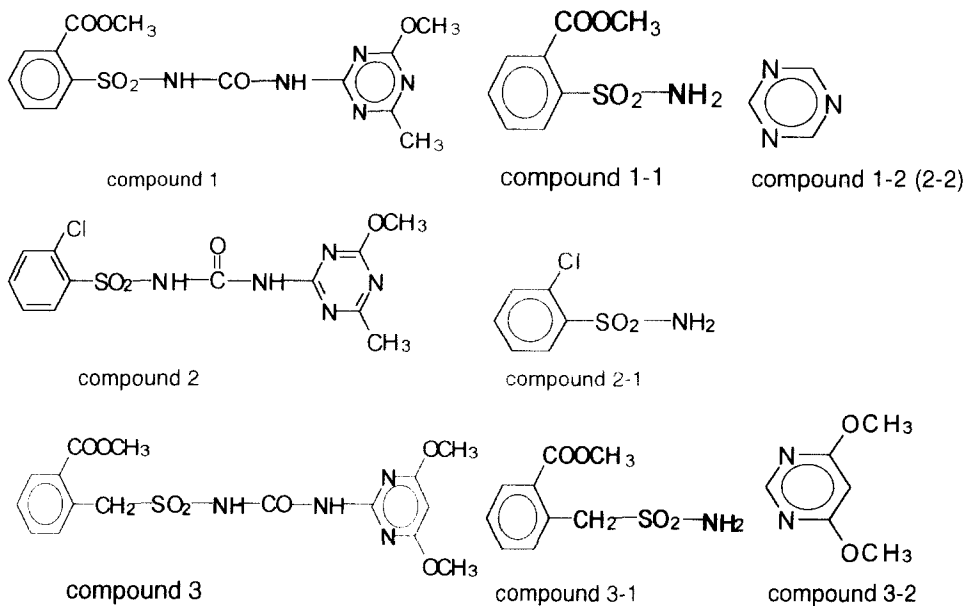
**To whom correspondence should be addressed.

factor (BCF), are related with hydrophobicity^[4], it also serves as basic information that can be correlated with biological activity.

In this paper the n-octanol/water partition coefficient (K_{ow}), the RP-HPLC capacity factors (K') and first order molecular connectivity index, of eight sulfonylurea herbicides and their degradation products, are used to describe their hydrophobicity. Their acute toxicity to the bacterium *Photobacterium phosphoreum* (15-min- $\log EC_{50}$, the concentration of a chemical that gives 50% illumination inhibition after 15-minute-exposure) and the green alga *C. pyrenoidosa* (96-h- $\log EC_{50}$, the concentration that gives 50% algal growth inhibition after 96-hour-exposure) was tested and estimated by $\log K_{ow}$ and $\log K'$. Their acute toxicity to the cladocera *Daphnia magna*, a primary consumer in fresh water ecosystem, was tested in laboratory and the result was discussed.

MATERIALS AND METHODS

Samples Three sulfonylurea herbicides (purity >95%), metsulfuron methyl (compd. 1), chlorsulfuron (compd. 2) and bensulfuron methyl (compd. 3), and their corresponding degradation products, compd. 1-1, 1-2, 2-1, 2-2, and 3-1, 3-2, were used. The chemical structures are as follow.



Determination of n-octanol/water Partition Coefficients The n-octanol/water partition coefficient was determined by the shake flask method as described by the OECD Guideline for Testing of Chemicals^[5] at 25 °C. Briefly, 1 mL sample solution in water-saturated n-octanol (100 mg/L) and 9 mL n-octanol-saturated water were pipetted into a 10 mL centrifuging tube, which then was shaken (end over end) for 2 hours, followed by centrifuging (2000 r/min. for 30 min.) and analysis of chemical in the aqueous phase with UV spectrometer against n-octanol-saturated water blank. To determine partition coefficients of compd. 1, 2 and 3, the water phase was acidified to pH 2 with HCl because of the acidic property^[6] of these chemicals, and was violently shaken for 10 minutes before subjected to centrifuging. The experiment was done in triplicate and $\log K_{ow}$ values were calculated.

Determination of RP-HPLC Capacity Factors A ODS-hypersil column, 10 cm × 4.6mm i. d., was used. Mobile phases were made by mixing methanol with water in the proportions of 95:5, 90:10, 85:15, 80:20, 75:25, 70:30 and 65:35 (v/v). Aqueous solution of sodium nitrate was used for the measurement of dead time, t_0 . The samples were prepared separately in methanol solutions. Retention times, t_R , were recorded, and capacity factor, K' , was calculated by $K'=(t_R-t_0)/t_0$ for each sample corresponding to different eluents. The measurements were at least duplicated. When to determine the retention times of compound 1, 2 and 3, mobile phase was acidified by mixing methanol with water containing 0.001 mol/L H_3PO_4 to convert the compounds from the ionic forms into molecular forms. The average reproductivity of each determination of retention time was better than 1.0% relative deviation.

Determination of 96-h Algal Growth Inhibition *Chlorella pyrenoidosa* used in this work was obtained from the Institute of Aquatic Organisms, Academia Sinica, Wuhan, PRC. *C. pyrenoidosa* was cultured in Bold Basal Medium (BBM) under germ-free conditions at $20 \pm 1^\circ C$ with illumination under fluorescent light (4500 lux, 24h/day) and regular shaking^[7]. In the test, portions (5mL each) of cell suspension (6×10^5 cells/mL) in the logarithmic phase of the growth curve were pipetted into five 100 mL culture vessels in which different amounts of the tested compound were dissolved in 25 mL germ-free BBM, and the cultivation was continued. After 96-hour exposure, the optical density of the cultures was measured at 680 nm on a spectrophotometer. Growth inhibition was calculated by dividing algal optical density by that of the controls. A linear correlation between logarithmic concentration and the inhibition rate was determined ($r > 0.90$). 96-h-logEC₅₀ (effective concentration of a tested chemical causing 50% algal growth inhibition) was then measured. Experiments were conducted in triplicate.

Determination of 15-min *Photobacterium phosphoreum* Bioluminescence Inhibition Toxicity *P. phosphoreum* (T₃ mutation) was supplied in the form of freeze dried powder by the Institution of Soil Science, Academia Sinica, Nanjing, PRC. *P. phosphoreum* was cultured in the yeast-tryptone-salts-glycerol broth at pH 7.0 ± 0.5 and $20 \pm 1^\circ C$ under germ free condition. Bioluminescence was measured by a Microtox[®] toxicity analyzer (Model DXY-2) made by the institute of Soil Science, Academia, Nanjing, PRC. In the test, portions (0.1mL each) of agitated cell suspension were transferred by syringe into five, 3-mL tubes in which different amounts of the tested compound were dissolved in 2 mL 3% NaCl solution, and shaken vigorously for 1 min. followed by exposure to air for 15 min. (accurate to second). Bioluminescence was measured after 15-min-exposure, and inhibition rate was calculated against the control. 15-min-logEC₅₀ (effective concentration of a tested chemical causing 50% bioluminescence inhibition) was measured by the regression equation between inhibition rate and logarithmic concentration. Experiments were done in triplication.

Determination of 24-h Immobility Toxicity to New-born-*Daphnia magna* *D. magna* was obtained from local surfacewater about 3 years prior to the experiment and cultured in 15 L glass carboy containing 10 L of nutrient medium. *D. magna* were fed the green algae were served as food source. 10 *D. magna* (<24h old) cultured in 250 mL glass beakers that contained 200 mL lab test solution (the solution was diluted with experiment water containing dissolved oxygen of 8.2 ± 0.5 mg/L at pH 7.5 ± 0.3 and a hardness of 115 ± 8.0 mg/L expressed as CaCO₃). Immobility was examined to determine the half effective concentration (24-h-logEC₅₀) by probing for movement of the tested animals after 24-h-exposure^[7,8]. The control mortality was less than 10% for all analyses. The tests were done at $21 \pm 2^\circ C$ and in triplicate.

The regression analysis was performed using the " Statgraphics" program (STSC. Inc.; 1987).

RESULT AND DISCUSSION

Calculation of RP-HPLC Capacity Factor Derived from the retention time of a compound in RP-HPLC, capacity factor K' reflects hydrophobicity in a chromatographic system. The linear relationship between $\log K'$ and methanol concentration (ϕ) in the mobile phase is given by Equation 1^[9].

$$\log K' = \log K'_w - S \phi \quad (1)$$

where $\log K'_w$ is the intercept which represents the capacity factor for a compound when pure water is used as eluent, S is the slope, and ϕ is the volume percentage of methanol in the eluent. Because $\log K'_w$ is independent of any specific organic modifier effects, and they were basically independent from column specifications^[9], $\log K'_w$ is better than $\log K'$ to characterize the hydrophobicity. The regression equation for each compound was listed in Table 1 and its corresponding $\log K'_w$ was obtained.

Table 1. Correlation between RP-HPLC Capacity Factors and Methanol Content in Methanol-water Eluent

compounds	regression equation	r	$\log K'_w$
1-1	$\log K' = 0.41 - 0.014 \phi$	0.976	0.41
2-1	$\log K' = 0.93 - 0.019 \phi$	0.976	0.93
3-1	$\log K' = 0.60 - 0.016 \phi$	0.961	0.60
1-2	$\log K' = 0.30 - 0.013 \phi$	0.952	0.30
3-2	$\log K' = 2.64 - 0.035 \phi$	0.960	2.64
1	$\log K' = 2.81 - 0.039 \phi$	0.940	2.81
2	$\log K' = 3.32 - 0.044 \phi$	0.950	3.32
3	$\log K' = 5.35 - 0.065 \phi$	0.955	5.35

Calculation of First-Order Valence Molecular Connectivity Index Molecular connectivity is often used in describing molecular structure. It is based solely on bonding and branching patterns, and Kier and Hall have developed it from early work of Randic and demonstrated their utility in prediction of bioactivity and physicochemical properties. It includes simple and valence connectivity indices in path and cluster levels. First-order valence connectivity index ($^1X^V$) was calculated according to the methods described by Kier and Hall^[10] and listed in Table 2.

Prediction of $\log K_{ow}$ with $\log K'_w$ and First-Order Valence Molecular Connectivity Index

The observed $\log K_{ow}$ and calculated $\log K'_w$ and X^V values were listed in Table 2.

Table 2. $\log K_{ow}$, $\log K'_w$, $^1X^V$ Values and the Predicted $\log K_{ow}$ Values of the Tested Compounds.

compounds	observed $\log K_{ow}$	$\log K'_w$	$^1X^V$	$\log K_{ow}$ predicted by Eq. 2
1-1	0.01	0.41	4.05	0.21
2-1	0.79	0.93	3.55	0.48
3-1	0.37	0.60	4.53	0.31
1-2	0.12	0.30	1.55	0.15
3-2	0.96	2.64	2.77	1.36
1	1.78	2.81	7.15	1.45
2	1.95	3.32	6.18	1.71
3	2.60	5.35	8.18	2.76

$\log K_{ow}$ values of the compounds tend to increase with the increasing of both $\log K'_w$ and ${}^1X^v$ values (Table 2). Regression equations, Equations 2 and 3, were obtained by linear regression between $\log K_{ow}$ and $\log K'_w$ and ${}^1X^v$.

$$\log K_{ow} = 0.516 \log K'_w \quad r = 0.984 \quad n = 8 \quad SE = 0.27 \quad (2)$$

$$\log K_{ow} = 0.25 {}^1X^v \quad r = 0.927 \quad n = 8 \quad SE = 0.56 \quad (3)$$

The equations show that partition coefficients are significantly and positively correlated with both capacity factors and connectivity index. The comparisons of the regression coefficient and the standard error between Equation 2 and 3 show that capacity factor method is more accurate than the connectivity index method for $\log K_{ow}$ prediction of these studied compounds. The predicted $\log K_{ow}$ values with Equation 1 were listed in Table 2 and they are well fitted with the observed values.

Prediction of Acute Toxicity to *P. phosphoreum* and *C. pyrenoidosa* with $\log K_{ow}$ and $\log K'_w$ Acute toxicity of the herbicides and their degradation products to the bacterium *P. phosphoreum* (15-min- $\log EC_{50}$) and to the green alga *C. pyrenoidosa* (96-h- $\log EC_{50}$) was listed in Table 3.

Table 3. Observed and Predicted 15-min- $\log EC_{50}$ to *P. phosphoreum* and 96-h- $\log EC_{50}$ to *C. pyrenoidosa*

compounds	15-min- $\log EC_{50}$ (mg/L)			96-h- $\log EC_{50}$ (mg/L)		
	observed	pred. by Eq. 4	pred. by Eq. 8	observed	pred. by Eq. 6	pred. by Eq. 9
1	1.52	1.73	1.65	-0.21	0.22	0.09
1-1	2.85	3.04	2.94	2.89	2.70	2.56
1-2	2.83	2.96	2.86	1.97	2.54	2.40
2	1.48	1.61	1.53	-0.39	-0.02	-0.14
2-1	2.20	2.47	2.37	2.06	1.60	1.47
2-2	2.83	2.96	2.86	1.97	2.54	2.40
3	1.18	1.13	1.05	-0.77	-0.93	-1.04
3-1	3.01	2.77	2.68	1.86	2.19	2.06
3-2	2.97	2.33		2.24	1.37	

The herbicides are more toxic to both *P. phosphoreum* and *C. pyrenoidosa* than their degradation products (Table 3). For example, 15-min- $\log EC_{50}$ values of the degradation products, compound 1-1, 1-2, 2-1, 3-1 and 3-2 are greater than compound 1, 2 and 3 by more than 1, and 96-h- $\log EC_{50}$ values are greater by 2 or 3. Simple linear regression analysis shows that the toxicity data are linearly correlative to $\log K_{ow}$ and $\log K'_w$, and the results are shown as follow.

$$15\text{-min-}\log EC_{50} = 3.05 - 0.74 \log K_{ow} \quad r = 0.92 \quad n = 8 \quad SE = 0.33 \quad (4)$$

$$15\text{-min-}\log EC_{50} = 2.95 - 0.34 \log K'_w \quad r = 0.80 \quad n = 8 \quad SE = 0.49 \quad (5)$$

$$96\text{-h-}\log EC_{50} = 2.71 - 1.40 \log K_{ow} \quad r = 0.93 \quad n = 8 \quad SE = 0.55 \quad (6)$$

$$96\text{-h-}\log EC_{50} = 3.05 - 0.74 \log K'_w \quad r = 0.84 \quad n = 8 \quad SE = 0.82 \quad (7)$$

With the regression coefficients better than 0.80, the linear relationships are significant. Equation 4 and 6 are better than Equation 5 and 7 because of the higher regression coefficient and lower standard error of estimation. The fitted lines for Equation 4 and 6 show that the point representing compound 3-2 is out of the 95% confidence of the estimated values, which means a different toxic mechanism of it from the other tested compounds. Improved regression equations, Equation 8 and 9, were arrived with the data of the compounds besides compound 3-2.

$$15\text{-min-}\log EC_{50} = 2.95 - 0.73 \log K_{ow} \quad r = 0.97 \quad n = 7 \quad SE = 0.19 \quad (8)$$

$$96\text{-h-logEC}_{50} = 2.57 - 1.39 \log K_{ow} \quad r = 0.96 \quad n = 7 \quad SE = 0.43 \quad (9)$$

The predicted toxicity values were obtained from Equation 4, 6, 8 and 9 and listed in Table 3, and they are well fitted with the observed values.

Toxicity to *D. magna* In our experiment we did not observe any significant acute toxicity of the herbicides and their degradation products to new-born *D. magna* even at a concentration approaching saturation, pH=7. This shows that the observed toxicity of the compounds to the bacterium *P. phosphoreum* and the green alga *C. pyrenoidosa* is higher than to the cladocera *D. magna*. Y.-H. Zhao et al [11] suggest that the difference can be attributed to different constitution of the species studied. The bacterium *P. phosphoreum* and the green alga *C. pyrenoidosa* are unicellular organism, and the obstruction of the cell wall to ionized form is smaller than that of *D. magna* tissues. Not only the nonionized form but also the ionized form could pass through the cell membrane to contribute to toxicity. In contrast with *P. phosphoreum* and *C. pyrenoidosa*, the permeability of plasma membrane of *D. magna* gill and other tissues (e. g. skin) constitutes a common barrier to adsorption and transfer of chemicals^[12]. The uptake of nonionized form is greatly faster than ionized form in *D. magna*^[13]. Only the nonionized fraction contributes significantly to toxicity. Therefore the toxicity of the compounds to *D. magna* is lower than toxicity to the bacterium *P. phosphoreum* and the green alga *C. pyrenoidosa*.

SUMMARY

We report the hydrophobicity and acute toxicity, to the bacterium *Photobacterium phosphoreum*, the green alga *Chlorella pyrenoidosa* and the cladocera *Daphnia magna*, of three sulfonylurea herbicides, metsulfuron methyl, chlorsulfuron and bensulfuron methyl, and their degradation products. Statistically significant quantitative structure-activity relationships, between n-octanol/water partition coefficient on one hand, and PR-HPLC capacity factor and first order connectivity index on the other, and between acute toxicity, to *P. phosphoreum* and *C. pyrenoidosa*, and hydrophobicity have been developed. They are predictive to rank potential hazardous of the herbicides and their degradation products. Due to ionic existence of the compounds they are of little toxic to *D. magna*.

REFERENCE

1. Saadi Hemmamda, Michelle Calmon & Jean P. Calmon. Kinetics and Hydrolysis Mechanism of Chlorsulfuron and Metsulfuron-Methyl, *Pestic. Sci.*, 40:71 (1994)
2. Bernhard M. Berger and N. Lee Wolfe, Hydrolysis and Biodegradation of Sulfonylurea Herbicides in Aqueous Buffers and Anaerobic Water-sediment Systems: Assessing Fate Pathways Using Molecular Descriptors, *Environ. Toxicol. and Chem.*, 15(9):1500 (1996)
3. J. Sabadie, Degradation of the Herbicides Chlorsulfuron on Various Dry Minerals, *Weed Research*, 32:429 (1992)
4. Hui Hong, Shuokui Han, Xiaorong Wang et al, Prediction of Partition Coefficient and Toxicity for Phenylthio, Phenylsulfinyl, and Phenylsulfonyl Acetates, *Environ. Sci. Technol.*, 29:3044 (1995)
5. OECD Guideline for Testing of Chemicals, OECD: Paris, 1981
6. Bayer, E. M., Duffy, M. J., Hay, J. V. & Schlueter, D. D. *Herbicides, Chemistry, Degradation and Mode of Action* ed. P. C. Kearney & D. D. Kaufman. Marcel Deller. Inc. New York, 117 (1987).

7. APHA-AWWA-WPCF, Standard Methods for Examination of Water & Waster Water, 15th Edition, American Public Health Association (1980)
8. A. L. Buikema et al, Aquatic Invertebrate Bioassays, ASTM Special Technical Publication, 1982
9. Synder L. R. , Dolan, J. W., Gant J. R., Gradient Elution in High-performance Liquid Chromatography Theoretical Basis for Reversed-phase System, *J. Chromatogr.*, 165:3 (1979)
10. Kier L. B. , Hall L. H., Derivation and Significance of Valence Molecular Connectivity, *J. Pharm. Sci.*, 70:583 (1981)
11. Y.-H. Zhao, X. Yuan, L.-H. Yang and L.-S. Wang, Quantitative Structure-Activity Relationships of Organic Acids and Bases, *Bull. Environ. Contam. Toxicol.* 57:242 (1996)
12. Barron M. G., Bioconcentration, *Environ. Sci. Technol.* 24:1612 (1990)
13. Blum D. J. W. , Speece R. E., Quantitative Structure-activity Relationships for Chemical Toxicity to Environmental Bacteria, *Ecotoxicol. Environ. saf.* 22:198 (1991)