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Solid-Phase Microextraction for the Evaluation of Partition Coefficients of a Chlorinated Dioxin and Hexachlorobenzene into Humic Substances

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We developed a method for the evaluation of the partition coefficients (K_{oc}) of hexachlorobenzene (HCB) and 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) into humic substances (HSs) by using solid-phase microextraction (SPME). In the aqueous solution containing HCB or HpCDD and HS, the unbound species of HCB or HpCDD were accumulated on the SPME fiber. Subsequently, HCB or HpCDD on the SPME fiber was directly analyzed by GC-ECD. When the concentration of organic carbon in HS ([OC]) was plotted against the ratio of [HCB] or [HpCDD] in the absence of HS to that in the presence of HS, linear relationships were observed. The slope of the line corresponded to the K_{oc} value. The log K_{oc} values for HCB and HpCDD evaluated were in the ranges of 3.9 – 4.9 and 5.9 – 7.2, respectively. These values were the same order as those in the literature, which were evaluated by other methods (*e.g.*, solubility enhancement, solid-phase extraction and dialysis). The relative standard deviations of the log K_{oc} values evaluated in this study were within 5%.

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Introduction

Humic substances (HSs), which are widely distributed in soil, sediment and aquatic environments, can influence the fates and behaviors of hydrophobic organic pollutants (HOPs).[1-5] The partition of HOPs into HS has been useful for the reduction of ecotoxicity of HOPs and has also been useful for better understanding of their transportations in the environment.[6-9] To quantify the partition of HOPs into HSs, researchers have evaluated the partition coefficients of HOPs into HSs (K_{oc}) by measuring the unbound and bound species of HOPs with HSs. Some methods have been reported on the speciation analyses of HOPs in the presence of HSs: *e.g.*, liquid-liquid extraction,[10-12] dialysis,[1,6,13,14] fluorescence quenching,[5,15-18] solubility enhancement[19-22] and solid-phase extraction.[9,23,24] However, there were some problems in these methods: *e.g.*, using large amounts of organic solvents, chemicals and sample solutions, and direct exposure of the analysts to hazardous chemicals. In particular, for highly toxic compounds such as chlorinated benzene and dioxins, simpler and safer methods have been required.

A solid-phase microextraction (SPME) has been known as a simple technique for the preconcentration and pre-separation of analytes prior to gas chromatography.[25] In the aqueous mixture of HOPs and HSs, the unbound species of HOP are accumulated on the SPME fiber, although the bound species with HS are remained in the aqueous solution. The concentrations of unbound species can be determined by gas chromatography. This method has a variety of merits: *e.g.*, avoiding organic solvents, and minimizing the sample volume and the exposure of toxic chemicals to the analysts. The K_{oc} values using the SPME have been evaluated in terms of a variety of HOPs, such as PCBs, organo-tin and polycyclic aromatic hydrocarbons.[26-30]

Because HSs can largely influence the fates and behaviors of polychlorinated dibenzo-*p*-dioxins (PCDDs) in soil and aquatic environments, the K_{oc} values of PCDDs for a variety of HSs should be available. Nevertheless, only a few K_{oc} values of PCDDs have been reported, because of the difficulties in handling PCDDs.[31-34] In the present study, to simplify the evaluation of K_{oc} for PCDDs, the analysis of the unbound species of PCDD was examined by using SPME, followed by use of a gas chromatograph with electron capture detection (GC-ECD). A 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) was selected as an example of PCDDs. For the comparison, the K_{oc} values of hexachlorobenzene (HCB) were also evaluated.

Experimental

Materials and reagents

The HSs were extracted and purified from the commercial product and four soils in Japan, according to a protocol of the International Humic Substances Society (IHSS).[35] The origins and results of elemental analyses are summarized in Table 1. HCB powder was purchased from Tokyo Kasei Kogyo, Co., LTD. (99% purity), and a stock solution was prepared by diluting it in acetone (5 mg l^{-1}). A standard solution of HpCDD (50 μg ml^{-1} in nonane) was purchased from Wellington Lab., and the stock solution was prepared by diluting it in acetone

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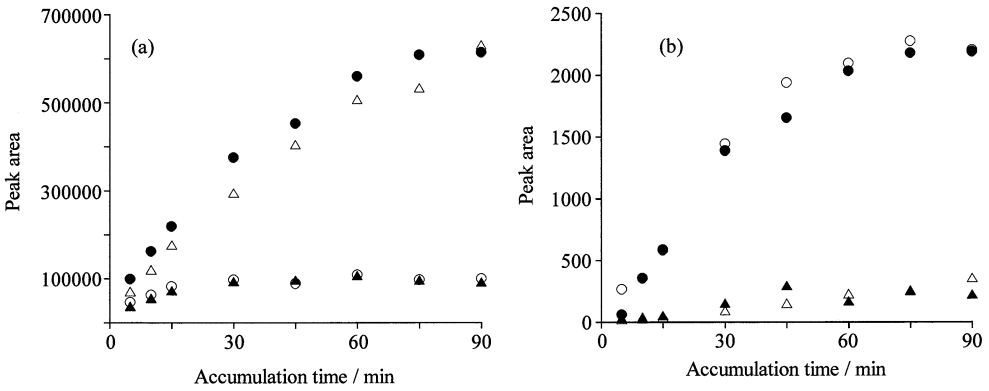

Fig. 1 Influence of accumulation time for SPME on the peak areas of HCB (a), and HpCDD (b) with different fiber thicknesses and extraction modes. ○, 7-μm fiber in the direct-extraction mode; ●, 100-μm fiber in the direct-extraction mode; ▲, 7-μm fiber in the headspace-extraction mode; and △, 100-μm fiber in the headspace-extraction mode.

Table 1 Origins and elemental analysis results for HSs

Origins of HS	%C	%H	%N	%O	%S	%Ash
Aldrich HA (AHA)	51.5	4.8	0.9	37.5	3.3	2.1
Tohro ando soil (THA)	49.3	4.6	1.1	38.9	2.6	3.6
Bibai peat soil (BHA)	50.6	5.4	2.8	37	0.6	3.3
Dando brown forest soil (DFA)	46.4	4.4	1.1	47.8	0.3	n.d.[a]
Inogashira ando soil (IFA)	45.4	4.4	2.1	46.0	0.4	1.5

a. Not detected.

(250 ng l^{-1}). These reagents were kept under cool and dark conditions. Acetone was purchased from Wako Pure Chemicals (special reagent grade). Pure water, which was used in the present study, was prepared by using a Millipore Nanopure System.

Optimization of SPME analysis

Aqueous solutions that contained HCB (100 ng l^{-1}) or HpCDD (2 ng l^{-1}), were prepared by diluting the stock solution with pure water. Each concentration of the aqueous solution was below water solubility of HCB (6200 ng l^{-1}) or HpCDD (2.4 ng l^{-1}). A 15 ml (headspace-extraction mode) or 18 ml (direct-extraction mode) aliquot of aqueous solution was placed in a 20-ml glass vial. Subsequently, each vial was sealed with a magnetic cap with a Teflon-faced septum. The 100- and 7-μm film thickness (these volumes were 0.026 and 0.61 μl, respectively) of polydimethylsiloxane (PDMS) fibers (1 cm length) were used with an automatic SPME holder (Supelco, Inc.). The fiber was exposed into the vial, and the aqueous solution was continuously agitated under an ambient temperature (25˚C). Two sampling modes, the direct- and headspace-extraction modes, were examined; the sampling time was varied in the range of 1 – 90 min. All operations were performed by means of a CTC Combi PAL SPME autosampler (CTC Analytics). The HCB or HpCDD that was accumulated on the SPME fiber was analyzed by means of an Agilent 6890A Type GC-ECD. The temperature of the ECD was set at 305˚C. The GC-ECD was equipped with a Quadrex Methylsilicon column (25 m × 0.25 mm i.d., 0.25 μm thickness). For HCB, the SPME fiber was desorbed at 295˚C for 6 min, during which the column temperature was at 60˚C. For HpCDD, the SPME fiber was desorbed in a splitless injector at 305˚C for 15 min, during which the column temperature was at 150˚C. The column temperatures then increased at 40˚C min^{-1} up to 120˚C and then 10˚C min^{-1} up to 300˚C (stationary for 10 min) for HCB, or increased at 40˚C min^{-1} up to 300˚C (stationary for 22 min) for HpCDD.

Partition equilibrium experiment with HOP into HS

The stock solutions of HS (1 g l^{-1}) were prepared by dissolving the HS powder in 0.1 N NaOH aqueous and then diluted with aqueous solution of 0.01 M KH_2PO_4/K_2HPO_4 buffer (pH 7.0). The concentrations of HS were set to 0 – 50 mg l^{-1} for HCB and to 0 – 1 mg l^{-1} for HpCDD. The stock solutions of HCB or HpCDD were added to each solution of HS to set the final concentration to 100 ng l^{-1} and 2 ng l^{-1}, respectively. The solutions were shaken at 500 rpm for 1 – 48 h. Subsequently, the unbound species of HpCDD or HCB in the solution were accumulated on the SPME fiber, and the concentrations were then determined by GC-ECD.

Results and Discussion

Optimization of analytical conditions

To optimize the analysis of HCB or HpCDD by the SPME method, we firstly investigated the influence of accumulation time. In this experiment, two sampling modes (direct- and headspace-extraction modes) and two types of fiber thickness (7- and 100-μm PDMS) were examined. Figures 1a and 1b show the influence of accumulation time on the peak area of HCB or HpCDD. In HCB (Fig. 1a), the peak areas for the 7-μm fiber (▲ and ○) were smaller than those for the 100-μm fiber (△ and ●). These results indicate that the peak areas depend on the fiber volumes, but not on the sampling modes. However, the peak areas for the 7-μm fiber had enough strength for the practical use. Because HCB is a volatile compound, the headspace-extraction mode is preferable. Therefore, 30 min of accumulation, 7-μm fiber and headspace-extraction mode were employed for the analysis of HCB.

In HpCDD (Fig. 1b), the peak areas obtained in the headspace mode (△ and ▲) were quite small and were not enough for practical use. This can be due to poor volatility of HpCDD. In the direct mode (○ and ●), larger peak areas were obtained than those in the headspace. The peak areas did not depend on the volumes of fibers. Therefore, 60 min of accumulation, 100-μm fiber, and direct-extraction mode were employed for the analysis of HpCDD. In HCB and HpCDD, the equilibration

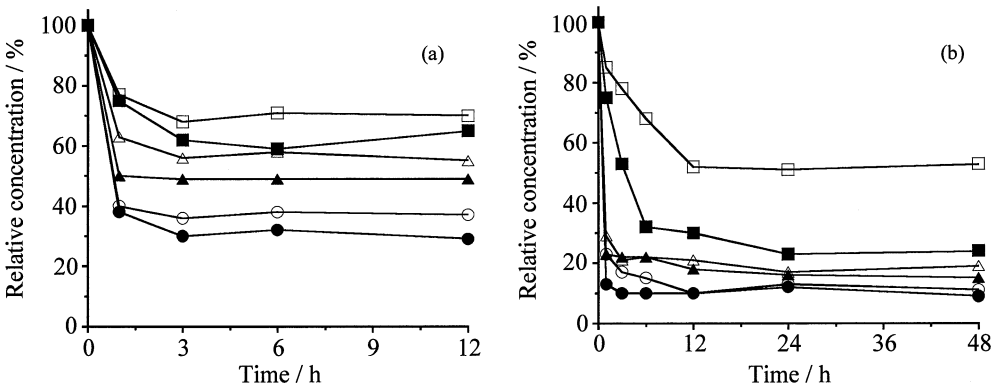

Fig. 2 Equilibrium kinetics of HCB (a), and HpCDD (b). (a): [AHA], 5 (□), 10 (■), 20 (△), 30 (▲), 40 (○), and 50 mg l^{-1} (●); sampling time, 30 min; fiber thickness, 7-μm PDMS; extraction mode, headspace. (b): [AHA], 0.1 (□), 0.2 (■), 0.4 (△), 0.6 (▲), 0.8 (○), and 1.0 mg l^{-1} (●); sampling time, 60 min; fiber thickness, 100-μm PDMS; extraction mode, direct. The concentrations of HCB and HpCDD in the absence of AHA were set to 100 as relative concentrations (%).

time for the 7-μm fiber was faster than that for the 100-μm fiber. Mayer *et al.* indicated that the rate constant of accumulation of analytes to the fiber increased with increasing the ratio of surface area to volume of polymer coating.[36] Such phenomena were also clearly observed in the present study.

In each optimized condition, the linear calibration curves for HCB and HpCDD were obtained in the concentration ranges of 0 – 100 ng l^{-1} (r^2 = 0.998) and 0 – 2 ng l^{-1} (r^2 = 0.999), respectively. The relative standard deviations of each data point in the calibration curves were 3.3 – 15.2% for HCB and 9.8 – 16.9% for HpCDD. The detection limits, which were calculated from 3σ values of blank peak areas, were 6.2 ng l^{-1} for HCB and 0.03 ng l^{-1} for HpCDD.

Equilibration time

To optimize the equilibration time for the partitions of HCB or HpCDD into HS, we investigated the equilibrium kinetics. Figures 2a and 2b show the kinetic curves of the partition equilibrium between HCB or HpCDD and AHA. In the absence of AHA, the concentrations of HCB and HpCDD did not vary during the entire equilibration period (data not shown). However, in the presence of AHA, the concentrations of unbound species of HCB ($[HCB]_w$) rapidly decreased in the initial 3 h and then reached a plateau (Fig. 2a). Moreover, the concentration of unbound species of HpCDD ($[HpCDD]_w$) rapidly decreased in the initial 6 h and then reached a plateau after 12 h of equilibration (Fig. 2b). Such tendencies for the equilibrium kinetics were consistent with the previous reports, in which HOPs such as PAHs are bound to HS over the short time periods.[29,37,38] Thus, the equilibration time of 3 h was determined for HCB and that of 12 h for HpCDD. As shown in Figs. 2a and 2b, the $[HCB]_w$ and $[HpCDD]_w$ decreased with increase in the [AHA]. These results indicate that the $[HCB]_w$ and $[HpCDD]_w$ are reduced by forming the bound species with AHA.

Evaluation of K_{oc}

In the previous reports,[2,20] the partition of HOPs into HSs has been considered as following this equilibrium:

$$\text{HOP} + \text{OC} \rightleftharpoons \text{HOP-OC} \quad (1)$$

where OC and HOP-OC represent the organic carbons in HS and the bound species of HOP with HS. In Eq. (1), K_{oc} can be defined as:

$$K_{oc} = \frac{[\text{HOP} - \text{OC}]}{[\text{HOP}]_w[\text{OC}]} \quad (2)$$

where $[HOP]_w$ represents the concentration of unbound species of HOP that can be accumulated to the SPME. The concentration of HOP in the absence of HS ($[HOP]_{total}$) is equal to the sum of $[HOP]_w$ and [HOP-OC]. Therefore, the following equation can be written:

$$[\text{HOP}]_{total} = [\text{HOP}]_w + [\text{HOP-OC}] \quad (3)$$

Combining Eqs. (2) and (3), one can derive the following relations:

$$\frac{[\text{HOP}]_{total}}{[\text{HOP}]_w} = 1 + K_{oc}[\text{OC}] \quad (4)$$

Equation (4) indicates that K_{oc} can correspond to the slope of the linear relationships between $[HOP]_{total}/[HOP]_w$ and [OC].

However, in the SPME method, the disturbance of partition equilibrium of HOPs between water and HS is troublesome during the extraction. It has theoretically been known that variation of the extractable concentration of the unbound species during the extraction could be kept less than 10% of the total concentration if the volume ratio of water to the fiber is much greater than the fiber-water partition coefficient (K_{SPME}).[29] In addition, it has been reported that this condition can be met even for highly hydrophobic PAHs.[39] It is also possible to suppose that such a condition was established in this study, because the volume ratios of water to the fiber (V_w/V_f) (HCB, 5.77×10^5; HpCDD, 2.95×10^4) were set similarly to those applied in the previous studies[29,40] (*e.g.*, 3.84×10^4, 9.62×10^5). As compared to the reported K_{SPME} value of HCB (1.91×10^5),[26] results indicate that the extractable HCB concentration could be less than 10% of the total and its partition equilibrium between water and HS would not be disturbed. The V_w/V_f ratio of HpCDD may be smaller than that of HCB, because HpCDD are more highly hydrophobic than HCB. Thus, more extraction could be expected for HpCDD.

In addition, if the partition equilibrium in the aqueous solution containing HS and HOP is not changed by the sorption of unbound species to the fiber, the $[HOP]_{total}/[HOP]_w$ ratio in the

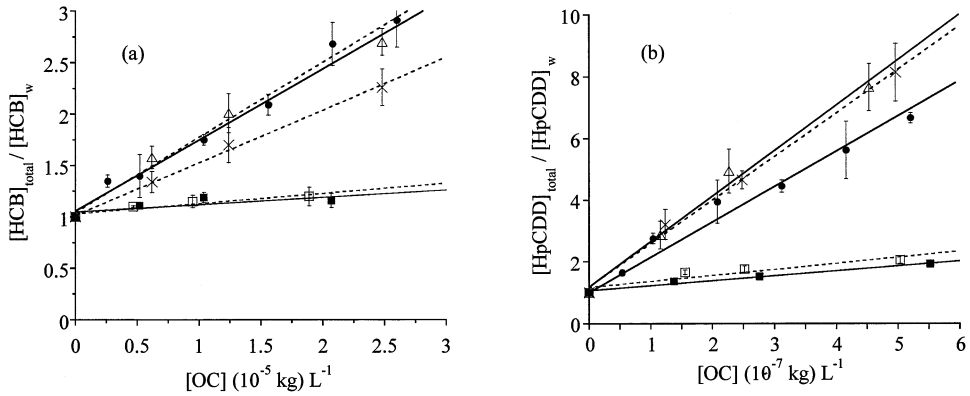

Fig. 3 Relationships between [OC] and $[HCB]_{total}/[HCB]_w$ (a), or $[HpCDD]_{total}/[HpCDD]_w$ (b). Types of HSs: AHA (●), THA (×), BHA (△), DFA (□), and IFA (■).

Table 2 The log K_{oc} values for HCB and HpCDD in a variety of HSs (n = 3 – 5)

HOP	log K_{oc} (present method)				log K_{oc} (literature value, method)
	HAs		FAs		
HCB	AHA	4.90 ± 0.04	DFA	4.13 ± 0.12	5.10, solid-phase extraction[9]
	THA	4.89 ± 0.07	IFA	4.11 ± 0.20	4.97 ± 0.05, SPME[26]
	BHA	4.73 ± 0.08			4.91, Dialysis[43]
HpCDD	AHA	7.13 ± 0.03	DFA	6.09 ± 0.14	5.5 – 7.8, solubility enhancement[31,32]
	THA	7.18 ± 0.07	IFA	6.31 ± 0.07	7.56, solubility enhancement[34]
	BHA	7.19 ± 0.03			

presence of HS could not be changed by the amounts of unbound HOP adsorbed on the fiber. Thus, the $[HOP]_{total}/[HOP]_w$ for 7-μm fiber was compared with that for 100-μm fiber. In HCB at [AHA] = 10 mg l^{-1}, the $[HCB]_{total}/[HCB]_w$ for the 7-μm fiber (1.31 ± 0.07, n = 4) was in good agreement with that for the 100-μm fiber (1.33 ± 0.29, n = 4). Moreover, in HpCDD at [AHA] = 1 mg l^{-1}, the $[HpCDD]_{total}/[HpCDD]_w$ for the 7-μm fiber (8.86 ± 0.55, n = 3) was also in good agreement with that for the 100-μm fiber (8.77 ± 0.95, n = 3). These results support the conclusion that the SPME method can extract the unbound species of HCB and HpCDD without disturbing the partition equilibrium.

Figures 3a and 3b show the relationships between [OC] and $[HOP]_{total}/[HOP]_w$ for HCB and HpCDD. As expected from Eq. (4), the linear relationships were observed, and the K_{oc} values were evaluated by the slope of each line. The log K_{oc} values are summarized in Table 2. For cases of both HCB and HpCDD, the log K_{oc} values were the same order as those in the literature that were evaluated by other methods.[9,26,31-34] The relative standard deviations of each log K_{oc} were within 5% (n = 3 – 5).

As shown in Table 2, the K_{oc} values for HpCDD were two orders of magnitude larger than those for HCB. These results are consistent with the logarithms of octanol–water partition coefficients (5.5 – 5.78 for HCB,[32,41,42] 8.2 for HpCDD).[22] Thus, these results reflect that the hydrophobic interaction includes the partition of HCB or HpCDD into HS. In addition, the K_{oc} values for HAs were one order of magnitude larger than those for FAs in HCB and HpCDD. These results probably reflect the structural differences between HAs and FAs, *e.g.* the atomic ratio of O to C (0.97 – 1.05 of HAs and 1.34 – 1.37 of FAs in this work) or organic C contents (49.3 – 51.4% of HAs and 45.5 – 46.4% of FAs).

Conclusion

To understand the fates and behaviors of PCDDs in soil and aquatic environments, evaluating the K_{oc} values should be required as an index of strength of binding between HSs and PCDDs. The SPME method, which is employed in the present study, can minimize the use of chemicals and the number of steps of procedures. Such merits contribute to the simple and rapid evaluation of the K_{oc} values of PCDDs for a variety of HSs as well as to the reduction of risks to the analysts.

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References

1. C. W. Carter and I. H. Suffet, *Environ. Sci. Technol.*, **1982**, *16*, 735.
2. C. T. Chiou, D. E. Kile, T. I. Brinton, R. L. Malcom, and J. A. Leenheer, *Environ. Sci. Technol.*, **1987**, *21*, 1231.
3. M. A. T. Jota and J. P. Hassett, *Environ. Toxicol. Chem.*, **1991**, *10*, 483.
4. B. E. Herbert, P. M. Bertsch, and J. M. Novak, *Environ. Sci. Technol.*, **1993**, *27*, 398.
5. N. A. Schlautman and J. J. Morgan, *Environ. Sci. Technol.*, **1993**, *27*, 961.
6. J. F. McCarty and B. D. Jimenez, *Environ. Sci. Technol.*, **1985**, *19*, 1072.
7. M. R. Servos, D. C. G. Muir, and G. R. B. Webster, *Aquatic Toxicol.*, **1989**, *14*, 169.

8. K. E. Day, *Environ. Toxicol. Chem*., **1991**, *10*, 91.
9. A. P. Freidig, E. A. Garikano, F. J. M. Busser, and J. L. M. Hermens, *Environ. Toxicol. Chem*., **1998**, *17*, 998.
10. E. T. Gjessing and L. Bergling, *Arch. Hydrobiol*., **1981**, *92*, 24.
11. G. Caron, I. H. Suffet, and T. Belton, *Chemosphere*, **1985**, *14*, 993.
12. S. Johnsen, *Sci. Total Environ*., **1987**, *67*, 269.
13. P. F. Landrum, S. R. Nihart, B. J. Eadle, and W. S. Gardner, *Environ. Sci. Technol*., **1984**, *18*, 187.
14. J. P. Hassett and E. Milicic, *Environ. Sci. Technol*., **1985**, *19*, 638.
15. R. B. Magee, L. Lion, and A. T. Lemley, *Environ. Sci. Technol*., **1991**, *25*, 322.
16. C. Rav-Acha and M. Rebhun, *Water Res*., **1992**, *26*, 1645.
17. K. D. Jones and C. L. Tiller, *Environ. Sci. Technol*., **1999**, *33*, 580.
18. J. Peuravuori, *Anal. Chim. Acta*, **2001**, *429*, 65.
19. T. Chiou, R. C. Malcom, T. I. Brinton, and D. E. Kile, *Environ. Sci. Technol*., **1986**, *20*, 502.
20. S. Tanaka, K. Oba, M. Fukushima, K. Nakayasu, and K. Hasabe, *Anal. Chim. Acta*, **1997**, *337*, 351.
21. F-D. Kopinke, A. Georgi, and K. Mackenzie, *Anal. Chim. Acta*, **1997**, *355*, 101.
22. Y-J. Kim, D-H. Lee, and M. Osako, *Chemosphere*, **2002**, *47*, 599.
23. H. J. M. Verhaar, F. J. M. Busser, and J. L. M. Hermens, *Environ. Sci. Technol*., **1995**, *29*, 726.
24. W. M. G. M. Van Loon, F. G. Wijnker, M. E. Verwoerd, and J. L. M. Hermens, *Anal. Chem*., **1996**, *68*, 2916.
25. C. Arthur and J. Pawliszyn, *Anal. Chem*., **1990**, *62*, 2145.
26. E. U. Ramos, S. N. Meijer, W. H. J. Vaes, H. I. M. Verhaar, and J. L. M. Hermens, *Environ. Sci. Technol*., **1998**, *32*, 3430.
27. J. Poerschmann, F-D. Kopinke, and J. Pawliszyn, *Environ. Sci. Technol*., **1997**, *31*, 3629.
28. F-D. Kopinke, J. Poerschmann, and M. Remmler, *Naturwissenschaften*, **1995**, *82*, 28.
29. J. Poerschmann, Z. Zhang, F-D. Kopinke, and J. Pawliszyn, *Anal. Chem*., **1997**, *69*, 597.
30. K. Mackenzie, A. Georgi, M. Kumke, and F-D. Kopinke, *Environ. Sci. Technol*., **2002**, *36*, 4403.
31. G. R. B. Webster, D. H. Muldrew, J. J. Graham, L. P. Sarna, and D. C. G. Muir, *Chemosphere*, **1986**, *15*, 1379.
32. D. Mackay, W. Y. Shiu, and K. C. Ma, in "*Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals: Polynuclear Aromatic Hydrocarbons, Polychlorinated Dioxins, and Dibenzo-furans*", **1992**, Lewis Publishers, Chelsea, MI.
33. K. Ballschmiter and R. Bacher, in "*Dioxin-Chemie, Analytik, Vorkommen, Umweltverhalten und Toxkologie der halogenierten Dibenzo-p-dioxine und Dibenzofurane*", **1996**, VCH, Weinheim.
34. Y. Kim and D. Lee, *J. Hazard. Mater*., **2002**, *91*, 113.
35. R. S. Swift, in "*Methods of Soil Analysis Part 3. Chemical Methods*", ed. D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnson, and M. E. Summer, **1996**, Soil Science Society of America, Madison, WI, 1018.
36. P. Mayer, J. Tolls, J. L. M. Hermens, and D. Mackay, *Environ. Sci. Technol*., **2003**, *37*, 185A.
37. D. A. Backhus and P. M. Gschwend, *Environ. Sci. Technol*., **1990**, *24*, 1214.
38. M. A. Schlautman and J. J. Morgan, *Environ. Sci. Technol*., **1994**, *28*, 2184.
39. J. J. Langenfeld, S. B. Hawthorne, and D. J. Miller, *Anal. Chem*., **1996**, *68*, 144.
40. W. H. J. Vaes, E. U. Ramos, H. J. M. Verhaar, W. Sinen, and J. L. M. Hermens, *Anal. Chem*., **1996**, *68*, 4463.
41. C. T. Chiou, D. W. Schmedding, and M. Manes, *Environ. Sci. Technol*., **1982**, *16*, 4.
42. A. T. Fisk, B. Rosenberg, C. D. Cymbalistry, G. A. Stern, and D. C. G. Muier, *Chemosphere*, **1999**, *39*, 2549.
43. C. G. Enfield, G. B. Bengtsson, and R. Lindqvist, *Environ. Sci. Technol*., **1989**, *23*, 1278.