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DETERMINING OCTANOL–WATER PARTITION COEFFICIENTS FOR EXTREMELY HYDROPHOBIC CHEMICALS BY COMBINING “SLOW STIRRING” AND SOLID-PHASE MICROEXTRACTION

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Abstract: Octanol–water partition coefficients (K_{OW}) are widely used in fate and effects modeling of chemicals. Still, high-quality experimental K_{OW} data are scarce, in particular for very hydrophobic chemicals. This hampers reliable assessments of several fate and effect parameters and the development and validation of new models. One reason for the limited availability of experimental values may relate to the challenging nature of K_{OW} measurements. In the present study, K_{OW} values for 13 polycyclic aromatic hydrocarbons were determined with the gold standard “slow-stirring” method (log K_{OW} 4.6–7.2). These values were then used as reference data for the development of an alternative method for measuring K_{OW}. This approach combined slow stirring and equilibrium sampling of the extremely low aqueous concentrations with polydimethylsiloxane-coated solid-phase microextraction fibers, applying experimentally determined fiber–water partition coefficients. It resulted in K_{OW} values matching the slow-stirring data very well. Therefore, the method was subsequently applied to a series of 17 moderately to extremely hydrophobic petrochemical compounds. The obtained K_{OW} values spanned almost 6 orders of magnitude, with the highest value measuring $10^{10.6}$. The present study demonstrates that the hydrophobicity domain within which experimental K_{OW} measurements are possible can be extended with the help of solid-phase microextraction and that experimentally determined K_{OW} values can exceed the proposed upper limit of 10^9. *Environ Toxicol Chem* 2016;35:1371–1377. © 2015 SETAC

Keywords: Octanol–water partition coefficient Polycyclic aromatic hydrocarbon Slow stirring Solid-phase microextraction Passive sampling

INTRODUCTION

The octanol–water partition coefficient (K_{OW}) is a very important parameter in the field of environmental chemistry and toxicology. Although its relevance for polar and in particular ionizable chemicals has been questioned, it is still widely used to predict sorption of nonpolar organic chemicals to soils, sediments, and dissolved organic carbon and to estimate bioaccumulation and effects of these chemicals [1]. Notwithstanding its importance, the availability of high-quality experimental data is relatively low. Many of the K_{OW} values being used for the above purposes are derived from models and, as such, associated with uncertainties, all the more because the models are often not sufficiently validated against experimental data. However, also experimental data suffer from uncertainty, in particular for hydrophobic chemicals, as illustrated by the large variation observed in experimental K_{OW} data sets [2].

The reason for this may be related to the fact that determining a high-quality K_{OW} is rather challenging. Almost 3 decades ago, it was concluded that shaking an octanol–water system may lead to biased K_{OW} values for the more hydrophobic chemicals because of the formation of a microemulsion of octanol in water. In response, the “slow-stirring” method was developed [3], which involves only gentle stirring of the octanol–water system. This method nowadays is considered the standard approach for determining K_{OW} [4,5]. However, even with this approach the determination of K_{OW} values for very hydrophobic organic chemicals is still challenging because of a number of practical and analytical issues. The aqueous concentration in an octanol–water system obviously decreases with the hydrophobicity of the test chemical. Measuring the resulting very low concentrations precisely is difficult not only because of analytical (detection limit) reasons but also practically because relatively large volumes of water need to be extracted using, for example, separation funnels, which is inconvenient. Also, the extraction of large water volumes results in the coextraction of a considerable volume of octanol (the aqueous solubility of octanol is ~0.5 g/L), which may lead to analytical issues later on during the instrumental analysis (e.g., peak deformation or interference with similarly boiling compounds, depending on the injection and detection technique). Because of the relatively high solubility of octanol in water and/or that of water in octanol, it has also been suggested that K_{OW} values may not exceed 9 on a logarithmic scale [6]. Very hydrophobic chemicals would be solubilized by octanol in water and/or experience a decreased chemical solubility in octanol because of the presence of water, resulting in an asymptotic approach of this limit.

To summarize, in particular for very hydrophobic chemicals, there is much uncertainty related to the existing K_{OW} values because the experimental determination of the parameter for these compounds is complicated. Consequently, there is a lack of high-quality experimental data, which hampers reliable chemical risk assessments as well as the validation of predictive models. The present study focused on the measurement of K_{OW} for very hydrophobic chemicals and aimed to 1) determine a high-quality K_{OW} data set for one of the most often studied groups of hydrophobic chemicals in environmental chemistry and toxicology, that is, 3-ring to 6-ring polycyclic aromatic hydrocarbons (PAHs), using the slow-stirring method (a consistent experimental data set for these compounds is still

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lacking); 2) use this new data set as a benchmark and develop an alternative approach for measuring K_{OW}, based on the use of solid-phase microextraction (SPME), trying to circumvent the above-mentioned practical and analytical issues associated with the slow-stirring method; and 3) apply the alternative approach to determine K_{OW} values for a set of moderately to extremely hydrophobic petrochemicals, having estimated log K_{OW} values of approximately 4.5 to 10. Based on the results of these determinations, the hypothesis [6] that experimentally determined K_{OW} values cannot exceed 10^9 was tested.

MATERIALS AND METHODS

Chemicals and SPME fibers

Testing chemicals included 13 PAHs and 17 petroleum hydrocarbons, of which the names, suppliers, and purities are given in Supplemental Data, Table S1. Other chemicals used were 2-methylchrysene (99.2%; BCR), PCB-31 (99%; Dr. Ehrenstorfer), and sodium azide (Merck). Solvents used were acetone, *n*-hexane, cyclohexane (Pestican grade; Lab Scan), methanol (high-performance liquid chromatography [HPLC] gradient grade; Lab Scan), acetonitrile (HPLC grade; Lab-Scan), and 1-octanol (99+%, HPLC grade; Aldrich). Polydimethylsiloxane-coated SPME fiber (glass fiber core diameter 110 μm, polydimethylsiloxane coating thickness 28.5 μm) was obtained from Poly Micro Industries. Prior to use, the fiber was cut into pieces of desired length, which were washed with methanol (3 × 20 min) and Millipore water (3 × 20 min).

Slow-stirring experiment

The slow-stirring experiment was performed with PAHs according to a previously described procedure [4,7], with some modifications. The determinations were performed for a PAH mixture, with each PAH at a single concentration, because octanol–water distribution is a partitioning process, being independent of the concentration or the presence of cosolutes [8]. Double-walled 1-L bottles were washed thoroughly with water, detergent, and solvents, and were air-dried. Then a metal (aluminum/nickel/cobalt) magnetic stirrer was added, and the bottles were filled with approximately 980 mL of Millipore water containing 25 mg/L of sodium azide. The water had equilibrated with octanol in an ultraclean 10-L bottle for 40 h, after which the water had been drained from a bottom tap. Next, 15 mL of octanol containing the 13 PAHs in appropriate concentrations (16–200 mg/L) was pipetted very carefully against the glass wall just above the water surface in the double-walled flasks, and the bottles were closed with glass stoppers. In total 8 flasks were prepared: 5 with octanol containing PAHs and 3 blanks (water + pure octanol). All systems were equilibrated by slowly stirring (creating a vortex of ~1 cm) for 3 wk at 20.4 ± 0.4 °C. Because the temperature was controlled tightly in the climate room, the double-walled flasks did not receive circulating, thermostated water. After 3 wk, which is more than sufficient to achieve full equilibration [4,7], a 1-mL octanol aliquot from each system was collected in an amber-colored autosampler vial and approximately 600 mL of the aqueous phase was drained from full-glass bottom taps in the double-walled flasks, using glass extension tubes. The first 100 mL was discarded, and the next 500-mL sample was collected in an ultraclean 1-L separation funnel standing in a support on a balance and containing 50 mL of *n*-hexane. This way, the exact weight of the aqueous phase was determined and PAH volatilization was prevented. All separation funnels were then placed on a mechanical shaker and shaken for 1 h. Once the phases were separated the extracted aqueous phase was collected in another separation funnel and extracted another 2 times in the same way with new 50-mL batches of *n*-hexane (half of each 50-mL batch was used first to rinse the previous separation funnel). All 3 *n*-hexane phases were pooled in pointed flasks and reduced in volume to 0.4 mL on a modified Kuderna-Danish apparatus and using nitrogen gas, successively. The *n*-hexane was finally exchanged to 0.45 mL of acetonitrile, 50 μL of an analytical internal standard (2-methylchrysene) solution was added, and the 0.5-mL extracts were transferred to amber-colored autosampler vials.

The aqueous-phase extractions were accompanied by recovery determinations ($n = 3$), which involved adding 100 μL of PAH spike (1.5 mg/L) to the *n*-hexane before the first extraction of 500 mL of pure Millipore water and comparing the results to measured concentrations in the spike. The extractions were performed in the absence of daylight (only reduced artificial light present) in order to minimize degradation of the photosensitive PAHs.

Octanol samples were diluted in acetonitrile (100 μL in 4.9 mL; masses recorded on a balance), and after homogenization, 100 μL of the solutions were diluted another 10 times in autosampler vials containing acetonitrile and internal standard.

Shake flask/SPME experiment

The K_{OW} values of PAHs were also determined with polydimethylsiloxane-coated SPME fibers in a miniaturized setup ($n = 7$). The use of these fibers was preferred over the use of, for example, polyoxymethylene (POM) or polyethylene (PE) as passive samplers because polydimethylsiloxane–water partition coefficients are lower than POM–water and PE–water partition coefficients, in particular for the more hydrophobic compounds [9,10]. For very hydrophobic chemicals, the required sampler–water partition coefficients are therefore "relatively" less challenging to determine in the case of polydimethylsiloxane. Also, equilibration times are expected to be longer for POM and PE samplers.

Scintillation vials (20 mL) were filled with 18 mL of Millipore water containing 25 mg/L of sodium azide, and 8 × 3 cm pieces of SPME fiber were added. Next, 2.0 mL of octanol containing PAHs (75–200 mg/L) was pipetted on top of the water, and the vials were closed and shaken on a reciprocal shaker (150 rpm) in the dark at 20 °C for 6 wk. Vials were then centrifuged (10 min at 3000 rpm) to separate the phases, and a 100-μL aliquot of octanol was sampled and diluted as described in the section *Slow-stirring experiment*. The remaining octanol was meticulously removed from the water surface by pipet. The fibers were then collected with tweezers, wiped clean with wet tissue, cut, and transferred to amber-colored autosampler vials, containing 180 μL of acetonitrile in a 250-μL insert. Finally, 20 μL of internal standard solution was added, and the vials were vortexed for 2 min.

Slow-stirring dual-flask/SPME experiment

The third approach that was used to determine K_{OW} values was a combination of the 2 methods described in the sections *Slow-stirring experiment* and *Shake flask/SPME experiment*: SPME fibers were applied to determine the aqueous concentration in systems that were slowly stirred. The objective was to avoid direct contact between the fibers and octanol as well as the formation of a microemulsion. To this end, special glassware was custom-made, consisting of 2 250-mL Scott-Duran bottles fused together via a glass tube (Figure 1). These dual-flask systems were filled with approximately 575 mL of Millipore water that

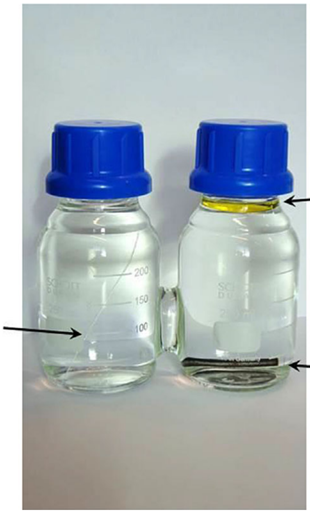

Figure 1. Photograph of a slow-stirring dual-flask/solid-phase microextraction system used for the determination of octanol–water partition coefficient values for polycyclic aromatic hydrocarbons and petroleum hydrocarbons. SPME = solid-phase microextraction.

had equilibrated for 40 h with octanol and contained 25 mg/L sodium azide. Fibers (2 × 10 cm) were placed in 1 bottle, and a metal magnetic stirrer and 5 mL of octanol were added to the second bottle. The second bottle was then stirred slowly, creating a vortex of approximately 1 cm. The octanol either contained PAHs (75–200 mg/L) or petroleum hydrocarbons (40–200 mg/L). In the first case, the systems were equilibrated in the dark at 20 °C for 2 wk ($n = 3$), 4 wk ($n = 3$), 6 wk ($n = 3$), or 8 wk ($n = 5$), after which the octanol and fibers were sampled or diluted, and extracted as described in the section *Shake flask/SPME experiment*. In the case of petroleum hydrocarbons, stirring lasted for 8 wk ($n = 3$), 14 wk ($n = 3$), 18 wk ($n = 4$), or 20 wk ($n = 4$); and fibers were extracted in cyclohexane instead of acetonitrile. Likewise, octanol was diluted in cyclohexane. For both PAHs and petroleum hydrocarbons, 3 blank systems were included, which were stirred for 6 wk (PAHs) or 18 wk (petroleum hydrocarbons).

Fiber–water partition coefficients

Fiber (polydimethylsiloxane)–water partition coefficients ($K_{PDMS\text{-}w}$) for PAHs required to derive the aqueous concentrations from concentrations measured in SPME fibers were determined in 5-fold as described before [11]. Briefly, 110 mL of Millipore water containing 25 mg/L sodium azide received 2 × 5 cm of fiber and was spiked with 25 μL of a 0.7-mg/L PAH cocktail spike in acetone. After 8 wk on a reciprocal shaker operating at 150 rpm, 100 mL of the aqueous phase was extracted 3 times with *n*-hexane, after which the pooled extract was concentrated, was exchanged to 0.45 mL of acetonitrile, and received 50 μL of internal standard solution. Final results were corrected for recoveries and blank determinations. Fibers were collected and extracted as described in the section *Shake flask/SPME experiment*. The $K_{PDMS\text{-}w}$ values for petroleum hydrocarbons were determined in a similar manner (see Supplemental Data for details). In addition, $K_{PDMS\text{-}w}$ values for PAHs were determined in octanol-saturated systems ($n = 5$). In this case, the aqueous phase had been saturated with octanol as described in the section *Slow-stirring experiment*, and fibers had been exposed for 3 wk in blank dual-flask systems with 575 mL of aqueous phase and 5 mL of pure octanol. Five replicate fibers were directly transferred to 200 μL of cyclohexane for determination of the concentration of octanol in polydimethylsiloxane, as described in the next section, *Chemical analysis*.

Chemical analysis

The PAH concentrations in all extracts and dilutions were determined with HPLC as described [12]. Petroleum hydrocarbons were quantified based on a 6-point calibration curve using a Thermo Trace GC Ultra (DSQ quadrupole) gas chromatograph mass spectrometer, equipped with a TriPlus autosampler and a Varian Factor Four capillary column (VF-5ms; 30 m × 0.25 mm × 0.25 μm) and operating in the selected ion mode. Details of the analysis are provided in the Supplemental Data. Octanol concentrations in fibers were determined on a Thermo Trace GC with a flame ionization detector and equipped with a TriPlus autosampler and an Agilent DB5.625 capillary column (30 m × 0.25 mm × 0.25 μm). Quantification was based on a 6-point calibration curve of octanol in cyclohexane.

Data analysis

The internal standard–normalized PAH concentrations in octanol (C_o) and water (C_w, corrected for blanks and recoveries) resulting from the slow-stirring experiment were divided in order to obtain K_{OW} values. For the experiments involving SPME measurements, concentrations in fibers (C_{PDMS}) were used to calculate the aqueous concentration by dividing by the respective $K_{PDMS\text{-}w}$ value. Then, K_{OW} values were derived as for slow-stirring experiments:

$$K_{OW} = \frac{C_o}{C_{PDMS}/K_{PDMS-w}} \tag{1}$$

RESULTS AND DISCUSSION

K$_{OW}$ *values for PAHs determined with slow stirring*

The K_{OW} values for PAHs as determined with the slow-stirring method are listed in Table 1. The presented values are all averages of 5 replicates and generally are accompanied by very small standard deviations. The quality of the data was assured by several precautions (see *Materials and Methods*), including the fact that all dilutions were made on a mass basis and the inclusion of multiple procedural blank and recovery determinations, for which the final data were adjusted (blanks were always <5%; recoveries varied between 95.1% and 99.8%, depending on the chemical). Also, all PAH quantifications were based on peak areas in the chromatograms, although in the author's laboratory PAHs are generally quantified based on peak height. This proved important because the presence of octanol in the water extracts caused peak broadening for mainly the 3-ring PAHs, which illustrates the analytical issues referred to in the *Introduction*. Quantifications based on peak height resulted in (biased) log K_{OW} values being up to 0.14 log units higher than values obtained based on peak area.

Considering the above, the data in Table 1 may be judged accurate and precise. This judgment is supported by the very good correlations ($r^2 \geq 0.99$) between the log K_{OW} values and other quality-controlled experimental "hydrophobicity data" (Figure 2A), that is, liposome–water partition coefficients [13], low-density PE–water partition coefficients [9], and silicone rubber–water partition coefficients [14]. Yet compared with the recommended K_{OW} values by Ma et al. [15], who recently critically reviewed the existing literature on physicochemical

Table 1. Averaged logarithmic octanol–water partition coefficients determined for polycyclic aromatic hydrocarbond using the slow stirring method and for petroleum hydrocarbons using the slow stirring dual-flask/solid-phase microextraction method[a]

	LogK_{OW} (L/L)
PAHs	
Phenanthrene	4.62 (±0.01; 5)
Anthracene	4.73 (±0.02; 5)
Fluoranthene	5.34 (±0.03; 5)
Pyrene	5.41 (±0.01; 5)
Benz[*a*]anthracene	5.97 (±0.00; 5)
Chrysene	5.95 (±0.01; 5)
Benzo[*e*]pyrene	6.58 (±0.01; 5)
Benzo[*b*]fluoranthene	6.50 (±0.02; 5)
Benzo[*k*]fluoranthene	6.63 (±0.02; 5)
Benzo[*a*]pyrene	6.69 (±0.02; 5)
Benzo[*ghi*]perylene	7.24 (±0.06; 5)
Dibenz[*a,h*]anthracene	7.22 (±0.04; 5)
Indeno[123,*cd*]pyrene	7.23 (±0.03; 5)
Petrochemicals	
Undecane	7.14 (±0.10; 8)
2,6-Dimethyldecane	8.03 (±0.11; 8)
2,6-Dimethylundecane	8.63 (±0.09; 8)
2,2,4,6,6-Pentamethylheptane	7.52 (±0.22; 8)
2,2,4,4,6,8,8-Heptamethylnonane	9.67 (±0.14; 8)
5,5-Dibutylnonane	10.60 (±0.25; 8)
n-Heptylcyclohexane	8.49 (±0.11; 8)
n-Octylcyclohexane	9.20 (±0.10; 8)
n-Heptylbenzene	6.03 (±0.04; 14)
n-Octylbenzene	6.69 (±0.06; 11)
n-Nonylbenzene	7.59 (±0.07; 8)
2-Iso-propylnaphthalene	4.81 (±0.03; 14)
2,6-Di-iso-propylnaphthalene	6.28 (±0.03; 14)
9-Methylanthracene	5.13 (±0.03; 14)
9,10-Dimethylphenanthrene	5.48 (±0.03; 14)
9,10-Dimethylanthracene	5.68 (±0.05; 14)
7,12-Dimethylbenz[*a*]anthracene	6.70 (±0.04; 14)

[a] Values in parentheses are standard deviations and number of replicates, respectively.

K_{OW} = octanol–water partition coefficient; PAH = polycyclic aromatic hydrocarbon.

PAH data, the currently presented values are relatively high, in particular for the high–molecular weight PAHs (Figure 2B; Supplemental Data, Table S2). Substantial differences of up to 0.6 log units to 0.8 log units for the 5-ring and 6-ring PAHs are observed. These differences may suggest that the literature values were biased because experimental artifacts associated with K_{OW} measurements typically result in underestimated values. The differences between the 2 sets may further be explained by the fact that the Ma et al. values do not originate from a single, consistent experimental data set, like the present study's data set, but are the outcomes of a mathematical adjustment procedure applied to the arithmetic mean of all collected values that were judged reliable by the authors. Especially in the absence of good reference values, however, judging the reliability of data is a difficult and subjective task. Moreover, in particular for the high–molecular weight PAHs, only few experimental data were available. Finally, the Ma et al. data apply to 25 °C, whereas the present values were determined at 20 °C. This temperature difference can, however, maximally explain a log K_{OW} difference of approximately 0.1 log units [16].

Because the data in Table 1 originate from a single, quality-controlled experiment, performed according to the gold standard (slow-stirring) method [5], one may consider them the best K_{OW} data available for the investigated PAHs. At least in the present study, they were therefore used as reference data for the development of an alternative, passive sampling–based approach for measuring K_{OW} values for very hydrophobic chemicals (see section K*OW values for petroleum hydrocarbons*). Also, the PAH log K_{OW} data from Table 1 were used to test the performance of several freely and/or easily available models/modeling software packages for predicting K_{OW}. These included the SPARC online calculator, the US Environmental Protection Agency's EPI Suite KOWWIN, ChemAxon's Marvin, and a poly parameter linear free energy relationship for K*OW* using data from the UFZ-LSER database (Helmholtz Centre for Environmental Research, Leipzig, Germany). In Figure 2C,D, the model estimates (all tabulated in Supplemental Data, Table S2) are plotted against the experimental values. Having a root mean square error of 0.12, SPARC clearly performs best. The experimental values are either perfectly or somewhat overpredicted by the online calculator. The 6-ring PAH dibenz[*a,h*]anthracene is, however, an outlier (overpredicted by 0.35 log units); and omitting the value for this chemical even results in a root mean square error of only 0.10. The worst predictions are performed by Marvin (root mean square error of 1.20). This modeling tool largely underestimates the experimental K_{OW} values, with the underestimation increasing with hydrophobicity. However, even for the 3-ring PAHs, the underestimation is considerable (0.7–0.8 log units). Also, the predictions clearly are based on molecular fragments but do not consider fragment positions because the same values are obtained for PAHs with the same number of rings (the exception is dibenz[*a,h*]anthracene again, for which a 0.3 log unit higher K_{OW} value compared to the other 6-ring PAHs is modeled). The poly parameter linear free energy relationship and KOWWIN perform intermediately, with root mean square errors of 0.37 and 0.44, respectively. Interestingly, the underpredictions by KOWWIN are rather constant, measuring approximately 0.4 log units to 0.5 log units, whereas the poly parameter linear free energy relationship predictions seemingly vary with the number of rings. The latter observation is surprising because poly parameter linear free energy relationships have recently been referred to as the most robust and accurate approach available for predicting equilibrium partition coefficients [17].

All in all, based on the above, the use of in particular Marvin for predicting K_{OW} values is discouraged. In contrast, SPARC appears to deliver reliable results, at least for PAHs; and this model was therefore selected for estimating K_{OW} values for the petroleum hydrocarbons (see section K*OW values for petroleum hydrocarbons*). The poly parameter linear free energy relationship (although statistically performing the second best) was not applied because the required compound descriptors appeared to be only available for 5 out of the 17 tested chemicals.

K*OW values for PAHs determined with SPME*

The above-discussed K_{OW} data set (in the section K*OW values for PAHs determined with slow stirring*) was used as a reference set for the development of an alternative K_{OW} determination method, attempting to reduce the labor intensity (i.e., mostly the liquid–liquid extractions of the water phase) and to circumvent the practical and analytical issues associated with the slow-stirring method. To this end, a simple miniaturized shake-flask setup in 20-mL vials combined with SPME was applied. Unfortunately, this method resulted in biased data, as discussed in the Supplemental Data; and therefore, it was considered not viable. Because the major problem with the shake flask/SPME setup seemed to be the direct contact between fibers and octanol, an alternative setup was applied in which this contact was

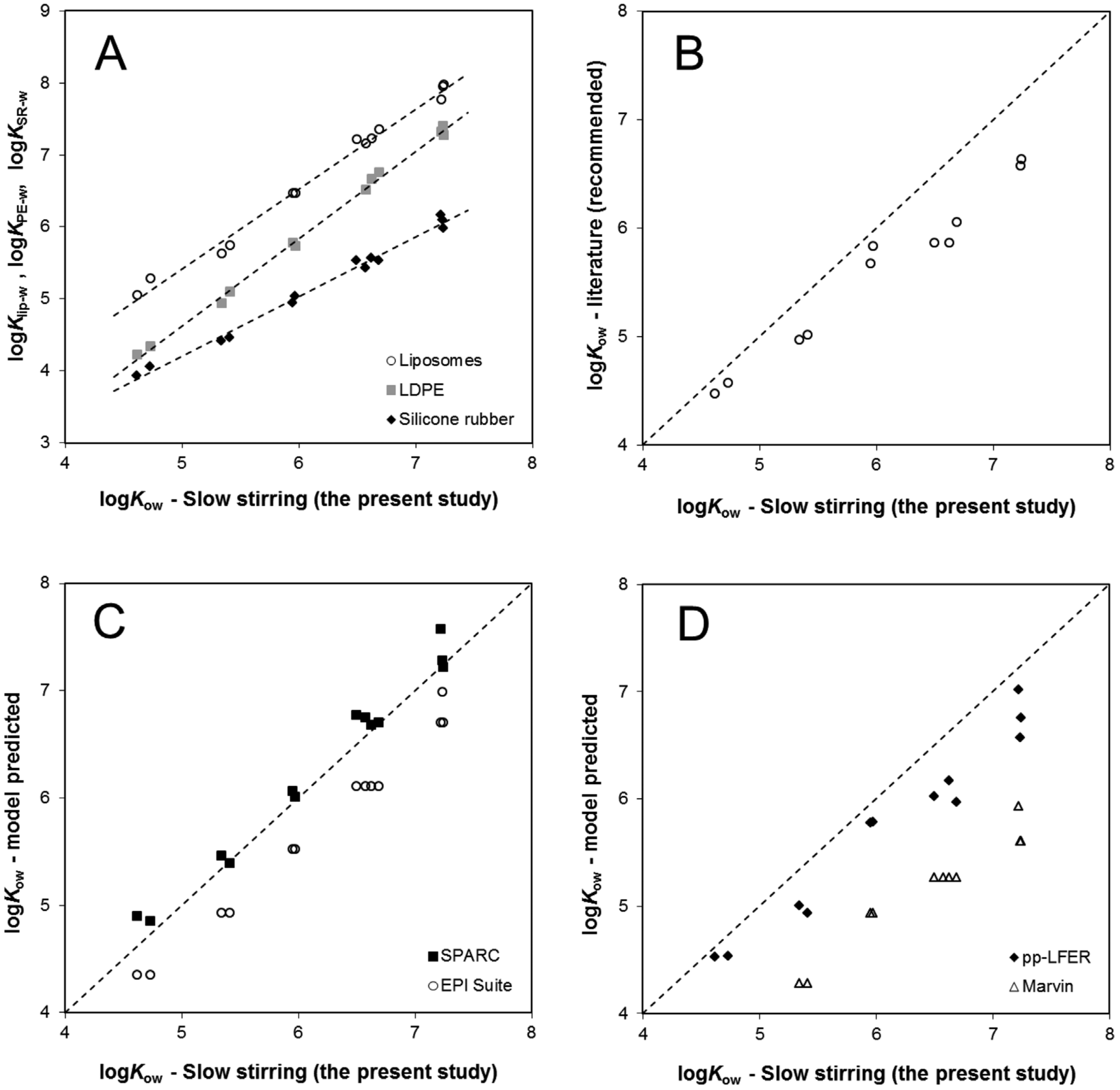

Figure 2. Relationships between polycyclic aromatic hydrocarbon octanol–water partition coefficient (K_{OW}) values determined with the slow-stirring method in the present study and (**A**) other hydrophobicity data: liposome–water partition coefficients (open circles [13]), low-density polyethylene–water partition coefficients (gray squares [9]), and silicone rubber–water partition coefficients (black diamonds [14]). Dashed lines are regression curves, all having $r^2 \geq 0.99$. (**B**) The final adjusted (recommended) K_{OW} values from Ma et al. [15]. (**C**) Model-predicted K_{OW} values by SPARC and EPI Suite KOWWIN. (**D**) Model-predicted K_{OW} values by a poly parameter linear free energy relationship for K_{OW} and Marvin. Dashed lines in graphs **B–D** represent the 1:1 relationship. $K_{lip\text{-}w}$ = liposome–water partition coefficient; $K_{PE\text{-}w}$ = low-density polyethylene–water partition coefficient; $K_{SR\text{-}w}$ = silicone rubber–water partition coefficient; LDPE = low-density polyethylene; pp-LFER = poly parameter linear free energy relationship.

avoided. The setup combined slow stirring with SPME in 2 conjoint flasks (slow-stirring dual-flask/SPME method; see *Materials and Methods* and Figure 1). Although equilibration of hydrophobic chemicals in octanol–water systems is achieved within a couple of days [4,7], equilibration times with this setup were 2 wk to 8 wk because equilibration of PAHs with the SPME fibers was expected to take several weeks. After all, the rate-limiting step in the equilibration process will be transport through the aqueous boundary layer surrounding the fibers, which probably is relatively thick in the slowly stirred systems. The results in Supplemental Data, Figure S3A demonstrate that 8 wk were actually required for the high–molecular weight PAHs, whereas 2 wk were sufficient for the low–molecular weight PAHs. After short exposure times, concentrations in the fibers were still low and applying equilibrium fiber–water partition coefficients ($K_{PDMS\text{-}w}$ values) in these cases will thus result in overestimated K_{OW} values (see Equation 1). As such, K_{OW} values resulting from this experimental method decrease and finally stabilize over time, as illustrated in Supplemental Data, Figure S3A. "Stabilized" K_{OW} values (i.e., the 8-wk data only [$n = 5$] for the 6-ring PAHs, up to the 2–8 wk [all] data [$n = 14$] for the 3-ring PAHs) were averaged, and the resulting data are presented in Supplemental Data, Table S3. In Figure 3, these averaged values are plotted against the slow-stirring data. The correlation between the 2 data sets is very good, but the slow-stirring dual-flask/SPME-derived K_{OW} values are on average 0.16 log units lower; and as a result of the small standard deviations for all data, values obtained by the 2 methods do significantly differ (t tests). The cause of this difference is unclear, but considering the absence of a trend with

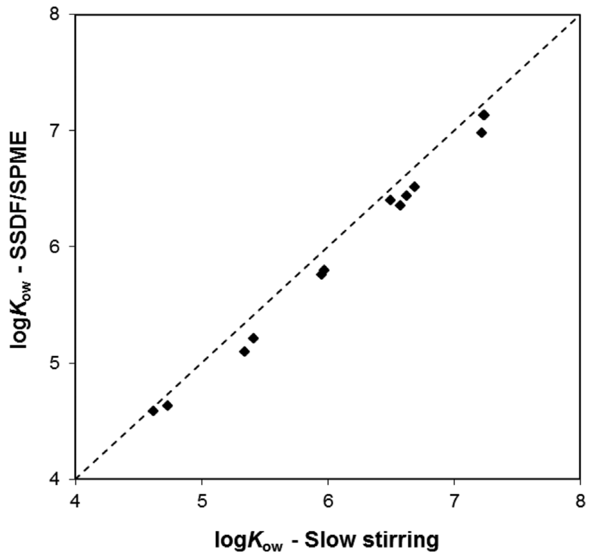

Figure 3. Relationship between octanol–water partition coefficient values for polycyclic aromatic hydrocarbons determined with the slow-stirring method and values determined with the slow-stirring dual-flask/solid-phase microextraction approach. K_{OW} = octanol–water partition coefficient; SSDF/SPME = slow-stirring dual-flask/solid-phase microextraction.

hydrophobicity, for instance, uncertainties in the applied $K_{PDMS\text{-}w}$ values (listed in Supplemental Data, Table S3) may play a role. Because the difference is, however, small, as a pragmatic approach it was considered marginal and the slow-stirring dual-flask/SPME setup was judged functional.

The observed close agreement between the slow-stirring data and the data obtained by using SPME demonstrates that in fiber–water systems containing octanol, one can simply apply $K_{PDMS\text{-}w}$ values that have been determined in pure water. This indicates that the presence of octanol in polydimethylsiloxane and water does not influence partitioning of the PAHs relatively (through either influencing the polydimethylsiloxane sorption capacity or cosolvent effects in the water phase). This is interesting because log $K_{PDMS\text{-}w}$ values determined in octanol-saturated systems (see section, *Fiber–water partition coefficients*) did significantly differ from values determined in pure water. Actually, the former did not make any sense because they were more or less independent of hydrophobicity, measuring 3.19 ± 0.12 on average (Supplemental Data, Figure S4). The cause of this hydrophobicity-independent partitioning behavior is not fully clear. Perhaps, although octanol was present in the aqueous phase only at its solubility level, the intensive shaking of the systems caused octanol to form micelles, which may have acted as a third phase. This could explain the absence of a normal slope with hydrophobicity, although rather a curvilinear relationship would be expected in such a case.

K_{OW} *values for petroleum hydrocarbons*

The slow-stirring dual-flask/SPME method was subsequently applied to a set of petroleum hydrocarbons. Because these included compounds being more hydrophobic than the 6-ring PAHs, equilibration times were extended up to 20 wk. The prolonged equilibration times proved necessary: whereas for the least hydrophobic petroleum hydrocarbons (i.e., the alkylated PAHs) K_{OW} values were constant over the full equilibration period (8–20 wk), for the more hydrophobic compounds values only stabilized at approximately 18 wk (Supplemental Data, Figure S3B). Nevertheless, for all compounds, equilibrium K_{OW} values could thus be derived. All equilibrium values were averaged, and the results (along with standard deviations and the number of replicates) are presented in Table 1. The experimental K_{OW} data set spans almost 6 orders of magnitude, with log K_{OW} ranging from 4.8 to 10.6. To the best of the author's knowledge, the data set thereby includes the highest experimentally determined K_{OW} values reported in the scientific literature so far.

In Figure 4, the data are plotted against SPARC-predicted K_{OW} values. The observed rather good correlation between the 2 data sets over 6 orders of magnitude, on the one hand, may suggest that the slow-stirring dual-flask/SPME experiment yielded reliable K_{OW} values (assuming that SPARC is as successful at predicting K_{OW} values for petroleum hydrocarbons as it is for PAHs, which is not unlikely considering the relative "simplicity" of the petroleum hydrocarbon molecules). On the other hand, it may illustrate the validity of the SPARC modeling tool. Figure 4 does suggest, however, that SPARC may have problems predicting values for specific chemicals because the 2 biggest outliers are both cyclohexanes (heptylcyclohexane and octylcyclohexane; measured log K_{OW} of 8.5 and 9.2, respectively, which are both underpredicted by SPARC by approximately a log unit).

The petroleum hydrocarbon K_{OW} data set also demonstrates that (experimental) log K_{OW} values can exceed 9 and are not limited by solubilizing effects of octanol in water and/or a reduced solubility in water-saturated octanol [6]. The highest measured value in the present study (i.e., 10.6 for 5,5-dibutylnonane) largely exceeds this limit, and log K_{OW} values for (branched) alkanes $>C_{17}$, for example, are actually expected to exceed it much further. The SPARC tool may be appropriate for predicting such values, although it should be stressed that extending the hydrophobicity range further will most probably lead to increased uncertainty in the predictions. The availability of a good model is essential though because experimental determinations will only be possible up to a certain hydrophobicity level. The exact position of this level is hard to define

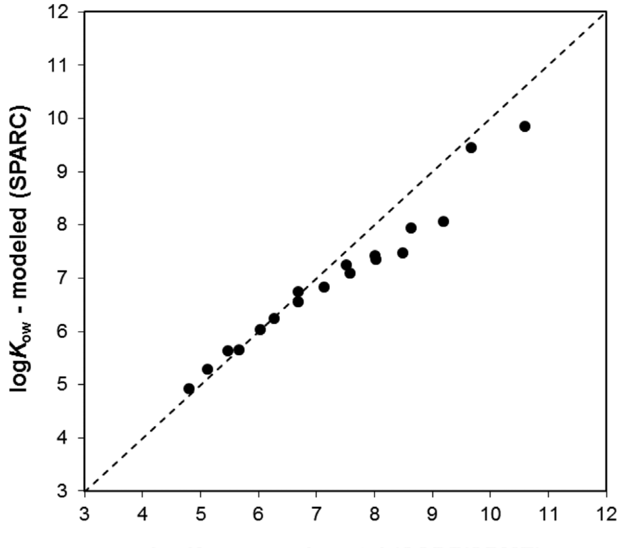

Figure 4. Relationship between octanol–water partition coefficient values for petroleum hydrocarbons predicted with SPARC and values experimentally determined with the slow-stirring dual-flask/solid-phase microextraction method. K_{OW} = octanol–water partition coefficient; SSDF/SPME = slow-stirring dual-flask/solid-phase microextraction.

because with the current slow-stirring dual-flask/SPME approach it depends on the possibility to determine $K_{PDMS\text{-}w}$ values. Basically, the main challenge in determining K_{OW} values for hydrophobic chemicals (i.e., measuring extremely low aqueous concentrations) is thus shifted to the challenge in determining $K_{PDMS\text{-}w}$. However, although during $K_{PDMS\text{-}w}$ determinations aqueous concentrations of very hydrophobic chemicals will also be very low, they will not be as low as during K_{OW} determinations. After all, polydimethylsiloxane–water partitioning is less strong compared with octanol–water partitioning (log $K_{PDMS\text{-}w}$ and log K_{OW} values diverge with increasing hydrophobicity; Supplemental Data, Table S3 and Muijs and Jonker [11]; cf. Table 1 and Supplemental Data, Table S4). Therefore, smaller water samples can be extracted, and coextraction of octanol and any subsequent issues do not play a role. Also important to note is that $K_{PDMS\text{-}w}$ values seem to level off at higher hydrophobicity (Supplemental Data, Table S4), whereas K_{OW} values do not, which might be explained by steric factors, that is, reduced uptake of very bulky compounds in polydimethylsiloxane. This phenomenon will cause an even larger divergence between log $K_{PDMS\text{-}w}$ and log K_{OW} and will thus actually increase the possibilities for measuring K_{OW} values of very hydrophobic chemicals with the slow-stirring dual-flask/SMPE approach. As an example, the highest log K_{OW} value measured in the present study for the branched C_{17} alkane 5,5-dibutylnonane (10.6) is based on a relatively low log $K_{PDMS\text{-}w}$ value of 7.1 as determined for this chemical (Supplemental Data, Table S4).

In summary, the slow-stirring dual-flask/SPME approach has some clear advantages compared to conventional slow-stirring determinations and may extend the hydrophobicity domain within which experimental determinations are possible. Still, the approach also does have obvious drawbacks. Most important, equilibrium times for very hydrophobic chemicals are long. In the present study's setup, times up to 18 wk to 20 wk were required. The equilibrium times are determined by the $K_{PDMS\text{-}w}$ values, as again illustrated by the 5,5-dibutylnonane case. This chemical has the highest K_{OW} value, but its relatively low log $K_{PDMS\text{-}w}$ value caused its equilibrium time to be similar to that for other chemicals having lower K_{OW} values but similar log $K_{PDMS\text{-}w}$ values. Thus, as mentioned in *K_{ow} values for PAHs determined with SPME*, chemical transport to the fibers is the rate-limiting step in the overall process. This step, and thereby the overall equilibration time of the system, could potentially be shortened by reducing the thickness of the aqueous boundary layer surrounding the fibers, by also stirring the compartment in which the fibers are placed (yet preventing contact between the stirrer and the fibers). Future experiments may demonstrate to what extent this will reduce the equilibrium times. Finally, it should be noted that the standard deviations of the log K_{OW} values for the most hydrophobic petroleum hydrocarbons are relatively large (Table 1). This is not inherent to the slow-stirring dual-flask/SPME approach, however, as demonstrated by the small standard deviations for the alkylated and parent PAHs (Table 1; Supplemental Data, Table S2). Rather, the increased variability is caused by the challenging behavior and analysis of the specific petroleum hydrocarbons, which also caused increased standard deviations in $K_{PDMS\text{-}w}$ values (Supplemental Data, Table S4) and liposome–water partition coefficients (unpublished data).

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3300.

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Data availability—Data, associated metadata, and calculation tools are available via the author (m.t.o.jonker@uu.nl).

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