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Estimating Phospholipid Membrane–Water Partition Coefficients Using Comprehensive Two-Dimensional Gas Chromatography

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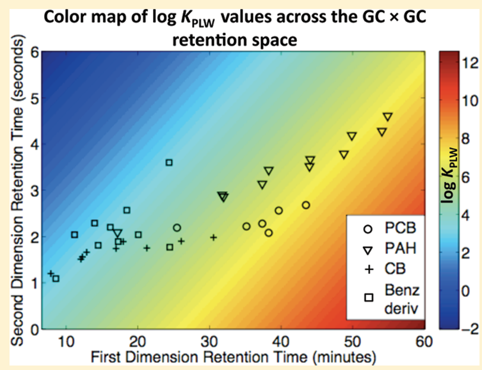
Supporting Information

ABSTRACT: Recent studies have shown that membrane–water partition coefficients of organic chemicals can be used to predict bioaccumulation and type I narcosis toxicity more accurately than the traditional K_{OW}-based approach. In this paper, we demonstrate how comprehensive two-dimensional gas chromatography (GC × GC) can be used to estimate such membrane–water partition coefficients (K_{PLW}s), focusing in particular on phosphatidyl choline based lipids. This method performed well for a set of 38 compounds, including polycyclic aromatic hydrocarbons, polychlorinated benzenes and biphenyls, and substituted benzenes including some phenols and anilines. The average difference between the estimated and the measured log K_{PLW} values of 0.47 log units is smaller than in the case of a log K_{OW} correlation approach but larger than seen using a polyparameter linear free energy relationship based approach. However, the GC × GC based method presents the advantage that it can be applied to mixtures of chemicals that are not completely identified, such as petroleum hydrocarbon mixtures. At the same time, our application of the GC × GC method suffered larger errors when applied to certain hydrogen bonding compounds due to the inability of the GC × GC capillary columns phases that we used to interact with analytes via hydrogen bond donation/electron acceptance.



INTRODUCTION

The bioaccumulation potential of organic contaminants is a key factor in environmental risk assessment of hydrophobic organic chemicals (HOCs). Compounds, for which the rate of biotransformation is slow compared to uptake, tend to accumulate in the lipids of exposed organisms, as dictated by their lipid–water or lipid–air partition coefficients.[1] One approach for calculating bioaccumulation relies on bioaccumulation/bioconcentration factors, obtained from linear free energy relationship correlations involving the *n*-octanol–water partition coefficient (K_{OW}).[2] This approach assumes that the partition properties of all types of lipids are the same, although recent studies[3,4] show that significant differences exist between the partitioning of chemicals into storage- versus membrane-lipids. In general, storage lipids consist of triacylglycerides (i.e., three aliphatic side-chains attached to a glycerol moiety). In contrast, membrane lipids are predominantly diacylglycerides (i.e., only two aliphatic side-chains attached to the glycerol unit), with a polar group attached at the third oxygen (Figure 1). This structural nature helps them form bilayers, a critical feature of biological membranes. For environmental contaminants such as phenols, Sandermann et al.[3] found that partitioning into storage lipids can be as much as a factor of 10 lower than the partitioning into membrane lipids, whereas for dichlorodiphenyltrichloroethane (DDT), partitioning into storage lipids was a factor of 10 higher than into membrane lipids. This could be explained, at least in part, by the fact that unlike the triacylgliceride storage lipids, the diacylglicerides have moieties that can function as electron density acceptors. Thus, in order to accurately predict bioaccumulation, both the phospholipid–water and triglyceride–water partition coefficients must be known. This is of particular importance in smaller organisms, such as plankton, in which the proportion of membrane-to-storage lipids is larger.[3] In addition, the correlation between the bioconcentration factor (BCF) and K_{OW} has been shown to break down for certain classes of compounds, including highly hydrophobic HOCs (log K_{OW} greater than 6),[5] and recent studies suggest membrane–water partition coefficients are better predictors for BCF than K_{OW}.[6]

The differential affinities of contaminants for the two different lipid classes are also relevant from a toxicological perspective. Membranes have been identified as the target site for nonspecific (or type I) narcosis toxicity.[7] Therefore, by knowing the partition coefficient of a particular contaminant into membrane lipids, one can calculate its activity at the target

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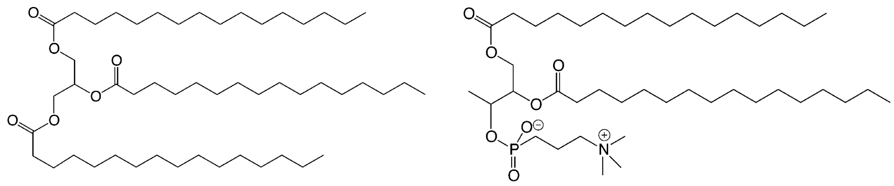

Figure 1. Examples of (left) a triglyceride storage lipid, tripalmitin ($C_{51}H_{98}O_6$, MW 807.34 g/mol), and (right) a membrane phospholipid, dipalmitophosphatidylcholine (DPPC, $C_{40}H_{80}NO_8P$, MW 734.04 g/mol).

site of toxicity and better evaluate the potential for toxic effects. Generally, such baseline toxicity has been obtained, similarly to BCFs, via a correlation against K_{OW}.[8] However, Vaes et al.[9] found that by using the membrane−water partition coefficient as a predictor instead of K_{OW}, (1) the relationship can be extended to a larger set of compounds, and (2) lethal body burdens (LBBs) can be better predicted by considering the differential partitioning into two separate lipid compartments.

Experimentally, determination of phospholipid−water partition coefficients (K_{PLW}) has largely focused on phosphatidyl cholines (PCs) with various side chains, as PCs readily form vesicles in water and because PCs are one of the most common components of membrane lipids in higher organisms.[10] These coefficients have been measured for a wide range of organic compounds including known environmental contaminants such as polychlorinated biphenyls (PCB),[4,6,11−12,13] polycyclic aromatic hydrocarbons (PAHs),[6,14,15] chlorobenzenes (CBs),[6,11,14,16,17] and other compound classes.[13,17] Unfortunately, for many compounds, there can be significant variability in the available data (e.g., K_{PLW} for PCB congener #155 (2,2′,4,4′,6,6′-hexachlorobiphenyl) varies by more than 2 orders of magnitude between ref 4 and ref 12). This may be due to experimental artifacts, but the data variation may also be caused by differences in the composition of aliphatic side chains or experimental temperatures that affect the phase of the lipids in question. At temperatures below the phase transition temperature (i.e., in the rigid gel phase), the aliphatic carbon atoms of the hydrophobic side chains reside primarily in the *anti* conformation; whereas above the phase transition temperature (i.e., in the liquid crystalline state), the *gauche* conformation becomes energetically favorable, leading to a more fluid, less well packed membrane.[18] Thus, the ability of the liposome to accumulate contaminants is higher in the liquid crystalline phase than in the rigid gel phase.[16] The value of the phase transition temperature is, in turn, affected by the nature of the lipid side chain, with smaller chains and higher degrees of unsaturation leading to lower transition temperatures.

In this study, we present a new method of estimating the K_{PLW} values of organic chemicals based on their retention behavior on a comprehensive two-dimensional gas chromatography system (GC × GC). Current experimental determinations of K_{PLW} values require phospholipid vesicles to be formed in a reproducible fashion and incubation experiments to be performed in such a way to ensure liposome stability and enough time for equilibration of highly hydrophobic compounds. In addition, K_{PLW} determinations are subject to variability due to exact nature of lipid and experimental temperature, as mentioned above. The method of estimating K_{PLW} values proposed here bypasses such methodological difficulties, and it can be applied when dealing with compounds for which experimental analysis is difficult.

Furthermore, the GC × GC method can be used to evaluate the potential for baseline (type I) narcosis toxicity of mixtures of chemicals, such as those in petroleum, which are not completely identified. Several studies have shown that components of the unresolved complex mixtures associated with petroleum contamination can cause baseline narcosis type I toxic effects in invertebrates, but a method is still needed to evaluate this toxicity. The high separation power given by GC × GC, coupled with the ability to give values of K_{PLW} for each of the mixture's components, which can in turn help us calculate the concentration of narcosis pollutants at their site of action, makes this technique ideal for estimating the cumulative baseline narcosis toxicity of such a mixture. The focus of this paper will be obtaining the values of K_{PLW} from a GC × GC chromatogram, while the application to mixture toxicity will be addressed in a subsequent paper.

Background. The advent of GC × GC has greatly improved our ability to separate and characterize components of complex organic mixtures; and recent studies show that with appropriate training sets, a range of physicochemical properties can be estimated from the retention behaviors of the analytes including their vapor pressures and octanol−water partition coefficients.[19] In GC × GC, the effluent from the first column is trapped, focused, and injected onto a second, shorter column at discrete time intervals. The stationary phase of the first dimension column is typically nonpolar (e.g., polydimethylsiloxane), resolving compounds based chiefly on their London dispersive interactions with the stationary phase (interactions dependent on a compound's molecular volume and polarizability). In the second dimension column, due to the presence of the phenyl groups (stationary phase is 50% phenyl polysilphenylene-siloxane, Figure S1), additional intermolecular interactions are also possible, notably hydrogen bond acceptance/electron density donation by the stationary phase. The apolar nature of the stationary phase mimics the partitioning from gas phase to a lipid phase, whereas the monopolar nature of the second dimension reflects some of the interactions that govern the partitioning from gas phase to water. Since K_{PLW} is a ratio of the lipid/gas and water/gas partition coefficients, then we expect that log K_{PLW} will be positively correlated with the first dimension retention time and negatively with respect to the second dimension. Mathematically this translates into a relationship of the following form for the calculation of log K_{PLW}

$$\log K_{PLW} = a^*RT_1 + b^*RT_2 + c \quad (1)$$

where RT_1 and RT_2 are the retention times of compound of interest in the first and second dimension, respectively.

Determination of partition coefficients, such as log K_{OW}, has been previously done using high pressure liquid chromatography and reverse phase column materials (HPLC). The advantage of this technique over GC is the presence of an aqueous phase, which directly captures the behavior of organic chemicals in water. However, in HPLC it takes a very long time to elute most compounds with only water; and if one uses an

Table 1. Experimental and Predicted log K_{PLW} Using Three Methods: GC × GC, Polyparameter Model[a], and log K_{PLW} = 1.01 log K_{OW} + 0.12[a]

no.	compound	experimental conditions[b]	log K_{PLW}[c]	this study	poly-param model	log K_{OW} fit
PCBs						
1	2-chlorobiphenyl (#1)	soy PC (25 °C)[d]	4.83	4.68	4.47	4.97
2	2,2′,5,5′-tetrachlorobiphenyl (#52)	soy PC (25 °C)[d], POPC (20 °C)[e], DMPC (26.5 °C)[e,f]	6.12	6.71	5.84	6.02
3	2,3,4,5- tetrachlorobiphenyl (#61)	soy PC (25 °C)[d]	7.15	7.01	6.24	6.59
4	2,2′,4,4′,6,6′-hexachlorobiphenyl (#155)	soy PC (25 °C)[d]	7.65	7.47	6.44	7.48
5	2,2′,3,3′,4,4′-hexachlorobiphenyl (#128)	soy PC (25 °C)[d]	7.88	7.71	7.13	7.02
6	2,2′,3,3′,6,6′-hexachlorobiphenyl (#136)	POPC (25 °C)[a]	6.50	7.16	6.67	7.31
PAHs						
7	naphthalene	egg PC (20 °C)[g]	3.38	3.09	3.58	3.52
8	phenanthrene	POPC (20 °C)[e,h], egg PC (20 °C)[g]	4.91	4.99	4.91	4.74
9	anthracene	POPC (20 °C)[e,h], egg PC(20 °C)[g]	5.04	5.08	5.00	4.71
10	fluoranthene	POPC (20 °C)[e,h]	5.68	5.81	5.51	5.39
11	pyrene	POPC (20 °C)[e,h], egg PC(20 °C)[g]	5.48	5.57	5.60	5.35
12	benz[*a*]anthracene	POPC (20 °C)[e,h]	6.53	6.64	6.37	6.04
13	chrysene	POPC (20 °C)[e,h]	6.49	6.44	6.38	6.09
14	benzo[*b*]fluoranthene	POPC (20 °C)[e,h]	7.23	7.25	6.62	6.22
15	benzo[*k*]fluoranthene	POPC (20 °C)[e,h]	7.24	7.25	6.82	6.22
16	benzo[*a*]pyrene	POPC (20 °C)[e,h]	7.37	6.85	7.00	6.08
17	dibenz[*a,h*]anthracene	POPC (20 °C)[e,h]	7.80	7.64	7.78	6.69
18	indeno[1,2,3-*cd*]pyrene	POPC (20 °C)[e,h]	7.97	7.62	7.25	6.69
19	benzo[*g,h,i*]perylene	POPC (20 °C)[e,h]	7.91	7.26	7.55	6.94
CBs						
20	chlorobenzene	DMPC (36 °C)[i], DMPC (26.5 °C)[f]	2.91	2.39	2.92	3.05
21	1,3-dichlorobenzene	DMPC (26.5 °C)[f]	3.71	2.81	3.58	3.69
22	1,4-dichlorobenzene	DMPC (26.5 °C)[f]	3.57	2.79	3.54	3.59
23	1,2-dichlorobenzene	DMPC (26.5 °C)[f], egg PC (20 °C)[g]	3.49	2.78	3.48	3.58
24	1,2,4-trichlorobenzene	DMPC (26.5 °C)[f]	4.20	3.52	4.12	4.21
25	1,2,3-trichlorobenzene	POPC (20 °C)[e], DMPC (26.5 °C)[e,f]	4.08	3.53	4.12	4.30
26	1,2,4,5-tetrachlorobenzene	POPC (20 °C)[e], DMPC (26.5 °C)[e,f], egg PC (20 °C)[g]	4.49	4.43	5.05	4.77
27	pentachlorobenzene	POPC (20 °C)[e], DMPC (26.5 °C)[e,f]	5.06	5.22	5.17	5.35
28	hexachlorobenzene	POPC (20 °C)[e], DMPC (26.5 °C)[e,f]	5.56	6.08	5.64	5.98
Miscellaneous						
29	*p*-xylene	DMPC (36 °C)[i]	2.98	2.71	3.12	3.30
30	aniline	DMPC (36 °C)[i]	1.63	1.96	1.59	1.03
31	nitrobenezene	DMPC (35 °C)[i], egg PC (20 °C)[g]	1.96	2.18	2.08	1.99
32	*N,N*-dimenthylaniline	DMPC (35 °C)[i]	2.33	2.97	2.55	2.45
33	2-nitrotoluene	DMPC (35 °C)[i]	2.41	2.76	2.55	2.44
34	2-allylphenol	DMPC (35 °C)[i]	3.06	3.42	N/A[j]	2.69
35	quinoline	DMPC (35 °C)[i]	1.67	2.79	2.12	2.44
36	4-chloro-3-methylphenol	DMPC (35 °C)[i]	3.34	3.79	3.10	3.25
37	*m*-nitroaniline	DMPC (35 °C)[i]	2.17	2.54	2.09	1.50
38	4-*n*-pentylphenol	DMPC (35 °C)[i]	4.31	5.12	N/A[j]	4.22

[a]Used pp-LFERs developed in ref 13 (based on V, S, A, B, and L for PCBs and based on E, S, A, B, and V for everything else, as recommended by ref 13). [b]Various lipids used: egg L-α-phosphatidylcholine (egg PC), 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), dimyristoylphosphatidylcholine (DMPC). [c]Average of log K_{PLW} values from the various experimental conditions. [d]Reference 4. [e]Reference 6. [f]Reference 11. [g]Reference 14. [h]Reference 15. [i]Reference 17. [j]Solute descriptors not available.

organic cosolvent, one has to train the system to correct for cosolvent effects. Also, compared to GC, LC is not as effective at separating complex mixtures, thereby limiting one's ability to examine such real world exposures to mixtures. Further, the detector response (e.g., absorbance or fluorescence) is not anywhere near as constant from analyte to analyte in HPLC as that seen with a flame ionization detector (FID); hence HPLC detectors do not allow as dependable a simultaneous quantification of mixture components as the FID when examining mixtures such as those in petroleum hydrocarbons. Such contaminant quantification, when combined with key physical chemical properties like K_{PLW} values of each eluting peak, should allow eventual estimation of integrated membrane doses from mixtures (the long-range goal of our work).

MATERIALS AND METHODS

Preparation of Solutions. Most of the compounds used in the training sets were purchased as mixtures or individual compounds from Ultra Scientific, Inc. with the exception of several benzene derivatives which were purchased as individual compounds from Sigma-Aldrich Co. Neat compounds were dissolved in dichloromethane, and stock solutions were diluted to appropriate levels for GC × GC − FID analysis (~1−10 ng/μL).

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Selection of Training Set Compounds. We selected known environmental contaminants such as PCBs, PAHs, and chlorobenzenes (CBs), as well as several structurally diverse benzene derivatives like phenols, anilines, and nitroaromatics previously found to cause narcosis.[9] We chose the values of K_{PLW} for which the experiments were performed at temperatures at which the liposomes were in the liquid crystalline phase, since biological membranes are mostly found in this state at environmental conditions (for example, transition phase temperature of egg phosphatidyl choline is -10 ± 5 °C[13]). Additionally, in the liquid crystalline phase, the K_{PLW} dependence with temperature is small, on the order of 0.1–0.2 log units per 10 °C,[6,13,16] whereas a sharp change in partition behavior occurs below the transition phase temperature (e.g., for chlorobenzene there is a 1.6 log units difference in K_{PLW} between the two lipid states).[16] After critically reviewing the available data (see Supporting Information, Table S2) and checking it for consistency, we obtained the training set displayed in Table 1. The average log K_{PLW} was used when multiple K_{PLW} values were available from different experimental setups. The regression of log K_{PLW} values against the two retention times (eq 1) was performed using the Data Analysis regression feature in Microsoft Excel 2004.

Analysis by GC × GC. GC × GC analyses were performed on an Agilent 7890A gas chromatograph, equipped with a 7683 split/splitless injector, two capillary gas chromatography columns, a quad jet modulator (LECO Corporation, St. Joseph, MI), and flame ionization detector. The samples were injected in splitless mode. The inlet temperature was set at 300 °C, and the purge valve was opened after 1 min. The carrier gas used was H_2, set at a flow rate of 1 mL/min throughout the run. Using sequential pentane injections at 10 min intervals, we determined that the breakthrough time through the second dimension column decreased by ~30% throughout the run (from 1.710 to 1.150 s), which indicates that the flow rate in fact speeds up throughout the run. First dimension separations were performed using a 100% dimethylpolysiloxane capillary column (Restek, RTX-1, 0.25 mm inner diameter, 0.25 μm film thickness, 27.5 m length), which was ramped from 40 °C (0.5 min hold), to 333 at 4.92 °C/min. Compounds exiting the first column were cryogenically trapped and reinjected (modulated) onto the second column at 6 s intervals, via the quad jet modulator. The cold jet was dry liquid N_2. The hot jet was set at 40 °C above the temperature of the first dimension oven. The second column was a 50% phenyl polysilphenylene-siloxane capillary column (SGE BPX-50, 0.10 mm ID, 0.25 μm thickness, 1.5 m length), and it was programmed from 55 °C (0.5 min hold) and ramped to 348 at 4.92 °C/min, which maintained a constant offset of 15 °C between the two columns throughout the run. The FID was set at 330 °C and sampled at 100 Hz. This relatively fast temperature program sacrificed part of the separation power, but a wide range of compounds (n-C_8 to n-C_{34} alkanes in the first dimension) were eluted in a time efficient manner, suited to processing large sample sets as well as complex mixtures such as spilled petroleum. The variability of the GC × GC retention times from run to run was very small (variations of less than 0.01 min in the first dimension and of less than 0.03 s in the second dimension observed throughout all the runs).

RESULTS

GC × GC System Check and Use of Retention Times To Estimate log K_{OW} Values. In order to test the validity of the GC × GC setup used, we first performed a regression of log K_{OW} against the two retention times. Based on the work done by Arey et al.,[19] GC × GC retention indices allow one to estimate log K_{OW} with a standard error of about 0.2 log units, using the same two stationary phases as the ones used in this study. However, we wanted to observe the consequences of using retention times instead of retention indices used by Arey et al.[19] We found that for a comparable training set (Figure S2, Table S1), consisting of only apolar and monopolar compounds (PAHs, PCBs, and CBs), there was a good correlation between log K_{OW} and retention times

$$\log K_{OW} = (0.165 \pm 0.006)^*RT_1 + (-1.33 \pm 0.09)^*RT_2 + (3.32 \pm 0.16) \quad (2)$$

$$N = 41,\ R^2 = 0.957,\ SE = 0.29$$

As shown by Arey et al.,[19] retention indices can be reproduced even when using two different instrument setups: different column lengths, temperature ramps, and gas flows, but that would not be the case for retention times. Thus, we can obtain comparable results by using retention times instead of indices, with the downside that we would have to retrain the relationship if the instrument or the setup (e.g., column lengths or carrier flows) is changed. However, the task of retraining only involves running a set of standard mixtures and performing the two-dimensional regression. In contrast, the calculation of retention indices of Arey et al.[19] is significantly more involved mathemathically and requires GC × GC-specific parameters as inputs, such as hold up times or phase ratios of the columns.

GC × GC-Based Estimation of K_{PLW} Values. The set of compounds used to assess our ability to find a relationship between K_{PLW} and retention times included six PCBs, 13 PAHs, nine CBs, and 10 benzene derivatives including phenols, nitroaromatic compounds, and anilines (Table 1). The regression using the reported K_{PLW} values of this training set and the GC × GC retention times was quite good

$$\log K_{PLW} = (0.208 \pm 0.010)^*RT_1 + (-1.42 \pm 0.16)^*RT_2 + (2.50 \pm 0.22) \quad (3)$$

$$N = 38,\ R^2 = 0.953,\ SE = 0.45$$

The statistics of this regression are clearly better (smaller standard error, and larger R^2) than if we use only the first dimension retention times. This can be seen by using the first retention times (RT_1 values) of our training set of compounds to find a fit as if we had used a one-dimensional GC equipped with the same capillary column as the first dimension of the GC × GC and operated in similar flow and temperature program conditions

$$\log K_{PLW} = (0.127 \pm 0.009)^*RT_1 + (1.22 \pm 0.30) \quad (4)$$

$$N = 38,\ R^2 = 0.843,\ SE = 0.80$$

To evaluate the effectiveness of the GC × GC-deduced correlation (eq 3), we refit the relationship (eq 1) withholding one of the 38 compounds and predicted the log K_{PLW} of the 38th compound, and we repeated this procedure for each compound in our training set. The predicted log K_{PLW} values (Table 1, Figure 2A) had an average deviation from the measured value of 0.47 or a factor of 3 in the K_{PLW} (calculated

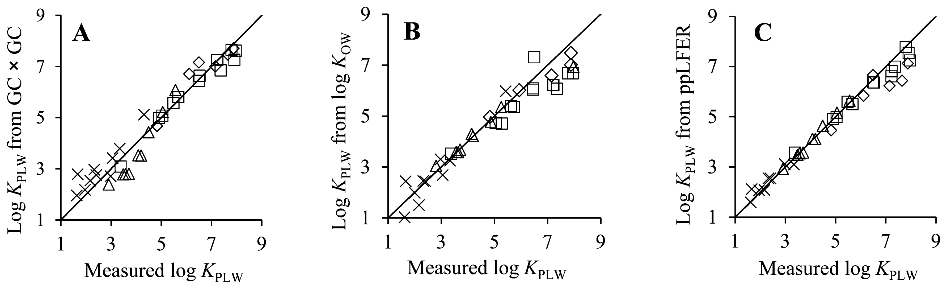

Figure 2. Comparison of log K_{PLW} predicted from GC × GC (panel A), using log K_{PLW} = 1.01*log K_{OW} + 0.12 (ref 13, Panel B) and polyparameter model[13] (panel C). Symbols represent compound classes: PCBs (diamonds), CBs (triangles), PAHs (squares), and miscellaneous (crosses). Also displayed in each panel is the 1:1 line.

as the square root of the sum of square deviations divided by number of observations minus 1). We noted that four of the divergent compounds were chlorinated benzenes which were all estimated too low, while three were hydrogen bonding compounds which were all overestimated (*N,N*-dimethylaniline, quinoline, *n*-pentylphenol). Suspecting that this bias may arise by inclusion of the polar compounds, we refit eq 1 excluding these seven compounds and found

$$\log K_{PLW} = (0.170 \pm 0.010)*RT_1 + (-0.984 \pm 0.146) *RT_2 + (2.71 \pm 0.16) \quad (5)$$

$$N = 29, R^2 = 0.969, SE = 0.30$$

The expression greatly reduced the chlorobenzenes' deviations from measured values (error now near 0.2 log unit) at the cost of no longer accurately estimating the polar compounds (error now near 1 log unit).

DISCUSSION

Comparison of GC × GC Method Versus ppLFER and log K_{OW} Approaches. Two other approaches have commonly been used to estimate K_{PLW} values. The first involves a correlation with octanol–water partition coefficients, and the second entails use of a polyparameter linear free energy relationship (ppLFER). To ascertain the relative accuracy of our new GC × GC approach, we contrasted estimates made in this way with those derived from these other methods. In each case, the K_{PLW} estimation method was applied, when possible to all the compounds in our GC × GC training set, and an average deviation between the estimated and the measured value of K_{PLW} was calculated as the square root of the sum of squared deviations divided by number of observations minus 1.

First, we compared the performance of the GC × GC-based method of estimating K_{PLW} to a linear free energy relationship (LFER) approach based on log K_{OW}. While many such relationships are available in the literature, we chose to use the one of Endo et al.[13] because it was developed using the largest number of compounds (log K_{PLW} = 1.01 log K_{OW} + 0.12; N = 156, SE = 0.426, R^2 = 0.948). This method of estimating K_{PLW} showed larger deviations than the GC × GC method (average deviation between estimated and measured log K_{PLW} of 0.58 vs 0.47). Also the approach using log K_{OW} increasingly underestimated log K_{PLW} values for the highly hydrophobic PAHs, while the GC × GC method did not do so (Figure 2A vs Figure 2B, Table 1). In addition, the K_{OW}-based method depends on the availability of accurate K_{OW} values, which for PCBs for example can vary substantially in the literature.[20]

We also compared the GC × GC-based method with results obtained using a polyparameter solvation model. In the polyparameter solvation model,[21] the partitioning between two media (log K), such as water and phospholipids, can be described in terms of five dimensions of solute–solvent interactions using a relationship of the form

$$\log K = e*E + s*S + a*A + b*B + v*V + c \quad (6)$$

The capital letters refer to the solute parameters: E (excess molar refraction and hence polarizability), S (polarity), A (hydrogen bond acidity), B (hydrogen bond basicity), and V (solute size), and the small letters reflect the differential interactions of the solutes in the two partitioning phases. Previous investigations[13] found that the best-fit interaction coefficients for phospholipid/water, olive oil/water, and octanol/water partitioning systems have similar signs and magnitudes implying that the same intermolecular interactions govern partitioning in these systems (Table 2).

We applied the polyparameter equation developed for log K_{PLW} by Endo et al.[13] to all the compounds in our training set (Table 1, Figure 2C) with the exception of 4-*n*-pentylphenol and 2-allylphenol for which solute descriptors were not found in the literature. When we do this, for these 36 compounds, we find an average deviation between the estimated and the measured log K_{PLW} of 0.38. This is lower than the average deviation of 0.46 obtained when the GC × GC-based method is used, for the same 36 compounds. The polyparameter model is especially able to better characterize the phenols, anilines, and nitroaromatic compounds (average deviation across the miscellaneous group of compounds of 0.23 compared to 0.59 in GC × GC approach). This is understandable because the polyparameter model takes into account a wider range of intermolecular interactions, such as the ability of compounds to donate electrons/accept hydrogens, while the stationary phases used in the GC × GC setup do not capture these interactions (Table 2).

This limitation is apparent when one applies the ppLFER approach to explain partitioning behavior in GC systems. For the stationary phases we used, best fit ppLFER coefficients have been determined[22] (Table 2), and these show similar l coefficient values reflecting similar London interactions for both stationary phases, but increased e, s, and especially a coefficients for the 50% phenyl phase of the second dimension column. However, what is most noteworthy is that for both of our stationary phases, b is zero (neither of the two stationary phases can donate hydrogens for H-bonding) and this is the case for all commercially available stationary phases at this time. But for phospholipid–water partitioning, b is nonzero and negative, as this term reflects the differential ability of water and

Table 2. Polyparameter Model Coefficients for Retention Behavior on Two Stationary Phases (Ref 22, p 100) Similar to the Ones Used in the GC × GC Setup of This Work, As Well As for Calculating log K_{OW}, log K_{PLW}, and log $K_{olive\ oil/water}$ (All from ref 13)

stationary phase or partition coefficient	*c*	*e*	*s*	*a*	*b*	*l*	*v*
polydimethylsiloxane, 121 °C[a]	−0.19	0.024	0.190	0.125	0	0.498	
polymethylphenylsiloxane, 121 °C[a]	−0.372	0.071	0.653	0.263	0	0.518	
log K_{PLW}, 25 °C[a,b]	1.46	−0.80	−1.14	−1.09	−4.22	1.64	
log K_{PLW}, 25 °C[c]	0.23	0.84	−0.75	0.28	−3.86		3.37
log K_{OW}, 25 °C[c]	0.09	0.56	−1.05	0.03	−3.34		3.81
log $K_{olive\ oil/W}$, 37 °C[c]	0.02	0.56	−0.98	−1.94	−4.46		4.22

[a] $\log K = c+e*E+s*S+a*A+b*B+l*L$, the capital letters refer to decriptors of the compounds, previously explained in the text, with the exception of *L* which is the log of solute gas−liquid distribution constant for hexadecane at 298 K. [b] Adapted from ref 13 to be in the same set of parameters as the pp-LFERs of the two stationary phases. [c] $\log K = c+e*E+s*S+a*A+b*B+v*V$.

phospholipids to donate protons/accept electrons to/from the compounds of interest. We note, however, that even with a stationary phase that is able to function as an H-donor, there will still be some limitations. We would not be able to apply this GC × GC method to compounds that decompose when heated to GC temperatures nor to compounds whose boiling points are a lot higher than the maximum operating temperature of the stationary phases.

However, both the polyparameter model as well as the GC × GC based method should be able to characterize equally well hydrophobic compounds like PCBs and PAHs. When applied to the PCBs in our training set, the polyparameter approach of Endo et al.[13] estimated log K_{PLW} with an average deviation of 0.79 log units. In comparison, the average deviation obtained for the K_{PLW} of PCBs via the GC × GC method was 0.42. One possible reason for this discrepancy may be the differences in the training sets. Interestingly, for the PCBs used in our training set and measured by Jabusch and Swackhamer,[4] there is an average difference of 0.8 log units between the experimental value of log K_{PLW} and the polyparameter model prediction, with the experimental value always being higher. In comparison, Endo et al.[13] used a different PCB data set in developing their polyparameter model, and, thus, the differences between the two estimation methods appear to stem from the large variability in the available data on K_{PLW} values of PCBs. As with other partition constants, such as K_{OW}, the K_{PLW} values for highly hydrophobic compounds like PCBs are difficult to measure due to low solubilities and long equilibration times.

Limitations of the GC × GC-Based Method. With our current choice of stationary phases in the GC × GC, we expected that we would not be able to characterize compounds with a strong electron donating (H^+ accepting) character as reflected in the polyparameter *bB* term. Neither of the two stationary phases can donate hydrogens (Table 2); yet, the correlation is able to predict compounds like PAHs (error 0.28) and PCBs (error 0.42) with nonzero *B* character, as well as some of the miscellaneous compounds (error 0.59), such as aromatic amines. One explanation could be that the contribution of the *bB* character to the partitioning into phospholipids is minor compared to the contributions of the other interactions. This applies to PCBs for which *B* ranges from 0.02 to 0.20[23] rendering a maximum contribution of 0.8 log units to the value of log K_{PLW}. The second possible explanation is that the *B* character correlates with another descriptor, such as *E* or *S*, which is true for PAHs (Figure S3). Arey at el.[19] reached a similar conclusion when trying to investigate which kind of information can be provided from the retention behavior of diesel hydrocarbons on the same stationary phases, as used in this study. Lastly, the compounds that exhibit large *B* values that do not correlate with either of the other descriptors should reflect large errors in log K_{PLW}. This is true for some of the miscellaneous compounds such as quinoline (Figure S3). In order to reduce the errors associated with our method, we would need to employ better stationary phases, which could capture compounds with hydrogen-accepting character, but such GC phases are not currently available.

Applications of the GC × GC-Based Method: Estimating K_{PLW} values for New Compounds. We applied this method to the prediction of K_{PLW} for a set of PCBs and organochlorine pesticides (OCPs) for which, to our knowledge, there are no available experimental data on K_{PLW}. We compared our estimate against the polyparameter model of Endo et al.[13] (Figure 3 and Table S3). For PCBs, the two methods agree only up to log K_{PLW} of around 6. Beyond that, the GC × GC method predicts consistently larger values than the polyparameter method, leading to an overall positive bias. This could be explained by the inability of the stationary phases to capture the hydrogen bond donation interaction, which has a negative contribution to log K_{PLW} or by the difference in training sets mentioned earlier. A similar trend is observed for the organochlorine pesticides (Table S3), with the exception of a group of OCPs (heptachlor epoxide, methoxychlor, dieldrin, eldrin, and endosulfan) which all contain one or more oxygen atoms. For these, the differences between the two predictive

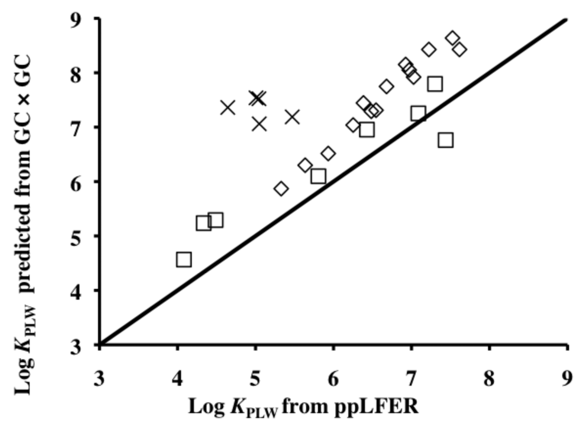

Figure 3. Comparison of K_{PLW} predicted from GC × GC and polyparameter model.[13] Symbols represent compound classes: PCB (diamonds), OCP (squares), oxygenated-OCP (crosses). Also displayed is the 1:1 line.

methods are on average 2.6 log units, most likely due to their pronounced hydrogen-bond accepting character (B terms larger than of all the compounds in the GC × GC training set).

Based on the results presented here, and the discussion on the limitation of the GC × GC method, we believe that this method can be accurately applied to compound classes such as petroleum hydrocarbons, PAHs, PCBs, and CBs, that is, hydrophobic chemicals commonly assessed for their likely impacts via type I narcosis toxicity. Larger errors are expected when this method is applied to compounds which can accept hydrogen bonds (based on our training set, compounds with B values greater than 0.4 tend to have deviations between the estimated and the measured log K_{PLW} greater than 0.5 log units). However, we note that the method may still work for B values greater than 0.4, if the B character is correlated with another descriptor (for example PAHs have B values greater than 0.4, but there is a correlation within the PAH family between the E and B descriptors, as shown also in Figure S3).

Applications of the GC × GC-Based Method: Estimating Baseline Narcosis Risks. The GC × GC method of estimating K_{PLW} values can also be applied to calculations of baseline (type I) narcosis toxicity of mixtures, e.g. petroleum hydrocarbon mixtures. For calculating baseline (type I) narcosis toxicity, we rely on two assumptions. First, we assume that all the components of the mixture partition independently into the membrane, contributing in an additive fashion to a type I narcosis effect.[24] Second, we assume that all the analytes quantified in the GC × GC run have virtually the same flame ionization detector (FID) response factor. Consequently, one could start with the GC × GC chromatogram of a passive sampler extract in which the concentration of each peak/compound can be calculated using the relatively constant response factor of the FID. In addition, at each point in the GC × GC space, one can calculate the value of the K_{PLW} and the passive sampler−water partition coefficient by using equations such as eq 1. The integrated dose of contaminants inside the membrane lipid then becomes a sum across the entire GC × GC space of all the calculated lipid concentrations of individual compounds. As opposed to other narcosis lipid models, such as the one proposed by McGrath et al.,[25] this approach would have the advantage that it does not require the identification of each single compound nor specific knowledge about their effect concentrations (i.e., the concentration required to produce a narcosis effect in 50% of the test organisms).

Applications of the GC × GC-Based Method: Estimating Bioaccumulation of Mixtures. For calculations of bioaccumulation, one would additionally require information about the proportion of storage versus membrane lipids and the value of the partition constant between the triglycerides and water (K_{TGW}) at each point in the chromatogram. The values of K_{TGW} could be calculated with a relationship of the form of eq 1, after running an appropriate training set of compounds with known K_{TGW} values on the GC × GC and finding the corresponding ppLFER regression coefficients. Then, assuming equilibrium with the environment, one could calculate the concentration of pollutants in each lipid compartment. One limitation of calculating bioaccumulation with this approach is that it would not apply to substances that are biotransformed at rates comparable to, or faster than, biouptake equilibration.

In this present study, we have shown that GC × GC retention behavior can be used to predict K_{PLW} for a series of chemicals within about a factor of 3. The results of the GC × GC-based method compared well with those from two other K_{PLW} prediction methods: a polyparameter LFER model and a log K_{OW}-based LFER. The practical advantages of predicting K_{PLW} from GC × GC retention behavior are (1) that it can be used to estimate K_{PLW} for compounds where experimental manipulations and analysis might be difficult (for example hydrophobic PCBs with long equilibration time scales, like those in our training set and Figure 3) and (2) that it can be applied to mixtures of hydrophobic chemicals that are likely to cause baseline narcosis toxicity (e.g., petroleum hydrocarbon mixtures) and for which separation and partitioning characterization of all the individual components might not be feasible. Compared to other methods of estimating partition coefficients, such as Arey et al.,[19] we have shown that GC × GC can be used for other compounds beyond hydrocarbons, such as PCBs, CBs, and some weak hydrogen bonding compounds, with the practical added simplification of using retention times instead of retention indices. Even though the relationship presented here between log K_{PLW} and retention behavior is valid only for the particular GC × GC setup used in this study, the method is easily transferable to other GC × GC systems, as it simply involves (a) running an HOC training set on the system in use at the site and (b) performing a simple regression on reported K_{PLW} values and the GC × GC retention times.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures S1-S3, text, and Tables S1-S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.

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