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



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# Estimating phospholipid membrane-water partition coefficients using comprehensive two-dimensional gas chromatography

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## 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.



**Keywords** Phospholipid-water partition coefficient ($K_{PLW}$), hydrophobic organic compounds (HOCs), comprehensive two-dimensional gas chromatography (GC × GC)

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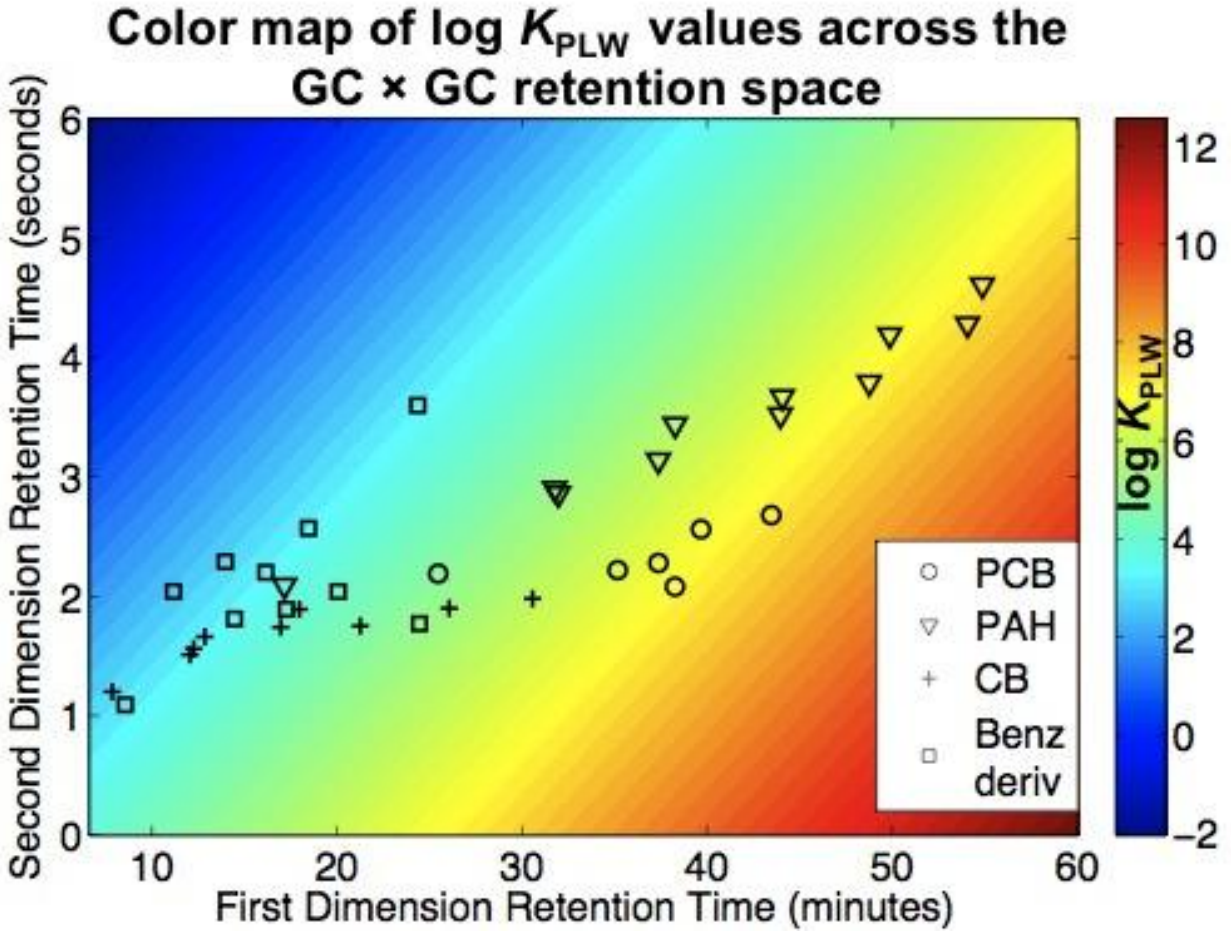


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40 INTRODUCTION

41 The bioaccumulation potential of organic contaminants is a key factor in environmental risk
42 assessment of hydrophobic organic chemicals (HOCs). Compounds, for which the rate of
43 biotransformation is slow compared to uptake, tend to accumulate in the lipids of exposed
44 organisms, as dictated by their lipid-water or lipid-air partition coefficients[1]. One approach for
45 calculating bioaccumulation relies on bioaccumulation/bioconcentration factors, obtained from
46 linear free energy relationship correlations involving the n-octanol-water partition coefficient
47 ($K_{OW}$)[2]. This approach assumes that the partition properties of all types of lipids are the same,
48 although recent studies[3-4] show that significant differences exist between the partitioning of
49 chemicals into storage- versus membrane-lipids. In general, storage lipids consist of
50 triacylglycerides (i.e., three aliphatic side-chains attached to a glycerol moiety). In contrast,
51 membrane lipids are predominantly diacylglycerides (i.e., only two aliphatic side-chains attached
52 to the glycerol unit), with a polar group attached at the third oxygen (Figure 1). This structural
53 nature helps them form bilayers, a critical feature of biological membranes. For important
54 environmental contaminants such as phenols, Sandermann et al.[3] found that partitioning into
55 storage lipids can be as much as a factor of ten lower than the partitioning into membrane lipids,
56 whereas for dichlorodiphenyltrichloroethane (DDT), partitioning into storage lipids was a factor
57 of ten higher than into membrane lipids. This could be explained, at least in part, by the fact that
58 unlike the triacylgliceride storage lipids, the diacylglicerides have moieties that can function as
59 electron density acceptors. Thus, in order to accurately predict bioaccumulation, both the
60 phospholipid-water and triglyceride-water partition coefficients must be known. This is of
61 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 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)[4,6,12-13], polycyclic aromatic hydrocarbons (PAHs)[6,14-15], chlorobenzenes (CBs)[6,11,14-17], and other compound classes[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 two orders of magnitude between ref. *4* and ref. *12*). This may be due to

85 experimental artifacts, but the data variation may also be caused by differences in the
86 composition of aliphatic side chains or experimental temperatures that affect the phase of the
87 lipids in question. At temperatures below the phase transition temperature (i.e., in the rigid gel
88 phase), the aliphatic carbon atoms of the hydrophobic side chains reside primarily in the *anti*
89 conformation; whereas above the phase transition temperature (i.e., in the liquid crystalline
90 state), the *gauche* conformation becomes energetically favorable, leading to a more fluid, less
91 well packed membrane[18]. Thus, the ability of the liposome to accumulate contaminants is higher
92 in the liquid crystalline phase than in the rigid gel phase[16]. The value of the phase transition
93 temperature is, in turn, affected by the nature of the lipid side chain, with smaller chains and
94 higher degrees of unsaturation leading to lower transition temperatures.

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

104 Furthermore, the GC × GC method can be used to evaluate the potential for baseline (type I)
105 narcosis toxicity of mixtures of chemicals, such as those in petroleum, which are not completely
106 identified. Several studies have shown that components of the unresolved complex mixtures
107 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 physico-chemical 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 organic co-solvent, one has to train the system to correct for co-solvent 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

153 *Preparation of solutions.* Most of the compounds used in the training sets were purchased as
154 mixtures or individual compounds from Ultra Scientific, Inc. with the exception of the several
155 benzene derivatives which were purchased as individual compounds from Sigma-Aldrich Co.
156 Neat compounds were dissolved in dichloromethane and stock solutions were diluted to
157 appropriate levels for GC × GC – FID analysis (~1-10 ng/μL).
158 *Selection of training set compounds*. We selected known environmental contaminants such as
159 PCBs, PAHs, chlorobenzenes (CBs), as well as several structurally diverse benzene derivatives
160 like phenols, anilines and nitroaromatics previously found to cause narcosis[9]. We chose the
161 values of $K_{PLW}$ for which the experiments were performed at temperatures at which the
162 liposomes were in the liquid crystalline phase, since biological membranes are mostly found in
163 this state at environmental conditions (for example, transition phase temperature of egg
164 phosphatidyl choline is -10±5 °C[13]). Additionally, in the liquid crystalline phase, the $K_{PLW}$
165 dependence with temperature is small, on the order of 0.1-0.2 log units per 10 °C[6, 13,16] , whereas
166 a sharp change in partition behavior occurs below the transition phase temperature (e.g., for
167 chlorobenzene there is a 1.6 log units difference in $K_{PLW}$ between the two lipid states)[16]. After
168 critically reviewing the available data (see Supplemental Information-1, Table S2) and checking
169 it for consistency, we obtained the training set displayed in Table 1. The average log $K_{PLW}$ was
170 used when multiple $K_{PLW}$ values were available from different experimental setups. The
171 regression of log $K_{PLW}$ values against the two retention times (Eq. 1) was performed using the
172 Data Analysis regression feature in Microsoft Excel 2004.
173 *Analysis by GC × GC.* GC × GC analyses were performed on an Agilent 7890A gas
174 chromatograph, equipped with a 7683 split/splitless injector, two capillary gas chromatography
175 columns, a quad jet modulator (LECO Corporation, St Joseph, MI), and flame ionization detector

(FID). The samples were injected in splitless mode. The inlet temperature was set at 300 °C and the purge valve was openned 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 minute intervals, we determined that the breakthrough time through the second dimension column decreased by ~30% throughout the run (from 1.710 seconds to 1.150 seconds), 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, 0.24 μm film thickness, 27.5 m length), which was ramped from 40 °C (0.5 min hold), to 333 °C at 4.92 °C/min. Compounds exiting the first column were cryogenically trapped and re-injected (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 °C 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:

$$\log K_{OW} = (0.165\pm0.006)*RT_1 + (-1.33\pm0.09)*RT_2 + (3.32\pm0.16) \qquad (2)$$

$$N = 41,\ R^2 = 0.957,\ \text{and SE} = 0.29$$

As shown by Arey et al.[19], retention indices can be reproduced by 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

222 phenols, nitroaromatic compounds, and anilines (Table 1). The regression using the reported
223 $K_{PLW}$ values of this training set and the GC × GC retention times was quite good:

224 $$\log K_{PLW} = (0.208\pm0.010)*RT_1 + (-1.42\pm0.16)*RT_2 + (2.50\pm0.22) \quad (3)$$

225 $$N=38,\ R^2 = 0.953,\ SE = 0.45$$

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

231 $$\log K_{PLW} = (0.127\pm0.009)*RT_1 + (1.22\pm0.30) \quad (4)$$

232 $$N=38,\ R^2 = 0.843,\ SE = 0.80$$

233 To evaluate the effectiveness of the GC × GC-deduced correlation (Eq 3), we re-fit the
234 relationship (Eq. 1) withholding one of the 38 compounds and predicted the log $K_{PLW}$ of the 38$^{th}$
235 compound, and we repeated this procedure for each compound in our training set. The predicted
236 log $K_{PLW}$ values (Table 1, Figure 2A) had an average deviation from the measured value of 0.47
237 or a factor of 3 in the $K_{PLW}$ (calculated as the square root of the sum of square deviations divided
238 by number of observations minus 1). We noted that four of the divergent compounds were
239 chlorinated benzenes which were all estimated too low, while three were hydrogen bonding
240 compounds which were all overestimated (*N*,*N*-dimethylaniline, quinoline, *n*-pentylphenol).
241 Suspecting that this bias may arise by inclusion of the polar compounds, we refit Eq. 1 excluding
242 these seven compounds and found:

243 $$\log K_{PLW} = (0.170\pm0.010)*RT_1 + (-0.984\pm0.146)*RT_2 + (2.71\pm0.16) \quad (5)$$

244 $$N=29,\ R^2 = 0.969,\ SE = 0.30$$

The expression greatly reduced the chlorobenzene's 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, Table 1). 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. 2B). 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:

$$\log K = e*E + s*S + a*A + b*B + v*V + c \quad (4)$$

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), $V$ (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 $K_{PLW}$ ppLFER 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

290 intermolecular interactions, such as the ability of compounds to donate electrons/accept
291 hydrogens, while the stationary phases used in the GC × GC setup do not capture these
292 interactions (Table 2).

293 This limitation is apparent when one applies the ppLFER approach to explain partitioning
294 behavior in GC systems. For the stationary phases we used, best fit ppLFER coefficients have
295 been determined[22] (Table 2), and these show similar *l* coefficient values reflecting similar
296 London interactions for both stationary phases, but increased *e*, *s*, and especially *a* coeffients for
297 the 50% phenyl phase of the second dimension column. However, what is most noteworthy is
298 that for both of our stationary phases, *b* is zero (neither of the two stationary phases can donate
299 hydrogens for H-bonding) and this is the case for all commercially available stationary phases at
300 this time. But for phospholipid-water partitioning, *b* is nonzero and negative, as this term
301 reflects the differential ability of water and phospholipids to donate protons/accept electrons
302 to/from the compounds of interest. We note however, that even with a stationary phase that is
303 able to function as an H-donor, there will still be some limitations. We would not be able to
304 apply this GC × GC method to compounds that decompose when heated to GC temperatures, nor
305 to compounds whose boiling points are a lot higher than the maximum operating temperature of
306 the stationary phases.

307 However, both the polyparameter model as well as the GC × GC based method should be able
308 to characterize equally well hydrophobic compounds like PCBs and PAHs. When applied to the
309 PCBs in our training set, the polyparameter equation (c) in Table 2 estimated log $K_{PLW}$ with an
310 average deviation of 0.79 log units. In comparison, the average deviation obtained for the $K_{PLW}$
311 of PCBs via the GC × GC method was 0.42. One possible reason for this discrepancy may be
312 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 non-zero *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 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

359 method is applied to compounds which can accept hydrogen bonds (based on our training set,
360 compounds with *B* values greater than 0.4 tend to have deviations between the estimated and the
361 measured log $K_{PLW}$ greater than 0.5 log units). However, we note that the method may still work
362 for *B* values greater than 0.4, if the *B* character is correlated with another descriptor (for example
363 PAHs have *B* values greater than 0.4, but there is a correlation within the PAH family between
364 the *E* and *B* descriptors, as shown also in Figure 3S).

365 *Applications of the GC × GC-Based Method: Estimating Baseline Narcosis Risks*

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

382 *Applications of the GC × GC-Based Method: Estimating Bioaccuulation of Mixtures*

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

392 In this present study, we have shown that GC × GC retention behavior can be used to predict
393 $K_{PLW}$ for a series of chemicals within about a factor of 3. The results of the GC × GC-based
394 method compared well with those from two other $K_{PLW}$ prediction methods: a polyparameter
395 LFER model and a log $K_{OW}$-based LFER. The practical advantages of predicting $K_{PLW}$ from GC
396 × GC retention behavior are (1) that it can be used to estimate $K_{PLW}$ for compounds where
397 experimental manipulations and analysis might be difficult, (for example hydrophobic PCBs
398 with long equilibration timescales, like those in our training set and Figure 3) and (2) that it can
399 be applied to mixtures of hydrophobic chemicals that are likely to cause baseline narcosis
400 toxicity (e.g. petroleum hydrocarbon mixtures), and for which separation, and partitioning
401 characterization of all the individual components might not be feasible. Compared to other
402 methods of estimating partition coefficients, such as Arey et al.[19], we have shown that GC × GC
403 can be used for other compounds beyond hydrocarbons, such as PCBs, CBs and some weak
404 hydrogen bonding compounds, with the practical added simplification of using retention times

405 instead of retention indices. Even though the relationship presented here between log $K_{PLW}$ and
406 retention behavior is valid only for the particular GC × GC setup used in this study, the method
407 is easily transferable to other GC × GC systems, as it simply involves (a) running an HOC
408 training set on the system in use at the site, and (b) performing a simple regression on reported
409 $K_{PLW}$ values and the GC × GC retention times.

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497
498 Table 1. Experimental and predicted log $K_{PLW}$ using three methods: GC × GC, polyparameter

499 model[a], and log $K_{PLW}$ = 1.01 log $K_{OW}$ + 0.12[a]

| # | 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 (25 °C)[e], DMPC (25 °C)[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)[g] | 3.71 | 2.81 | 3.58 | 3.69 |
| 22 | 1,4-dichlorobenzene | DMPC (26.5 °C)[g] | 3.57 | 2.79 | 3.54 | 3.59 |
| 23 | 1,2-dichlorobenzene | DMPC (26.5 °C)[g], egg PC (20 °C)[g] | 3.49 | 2.78 | 3.48 | 3.58 |
| 24 | 1,2,4-trichlorobenzene | DMPC (26.5 °C)[g] | 4.20 | 3.52 | 4.12 | 4.21 |
| 25 | 1,2,3-trichlorobenzene | POPC (20 °C)[e], DMPC (36 °C)[i], egg PC (20 °C)[g] | 4.08 | 3.53 | 4.12 | 4.30 |
| 26 | 1,2,4,5-tetrachlorobenzene | DMPC (26.5 °C)[g] | 4.49 | 4.43 | 5.05 | 4.77 |
| 27 | pentachlorobenzene | DMPC (26.5 °C)[g] | 5.06 | 5.22 | 5.17 | 5.35 |
| 28 | hexachlorobenzene | DMPC (26.5 °C)[g] | 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 (36 °C)[i], egg PC (20 °C)[g] | 1.96 | 2.18 | 2.08 | 1.99 |
| 32 | *N,N*-dimenthylaniline | DMPC (36 °C)[i] | 2.33 | 2.97 | 2.55 | 2.45 |
| 33 | 2-nitrotoluene | DMPC (36 °C)[i] | 2.41 | 2.76 | 2.55 | 2.44 |
| 34 | 2-allylphenol | DMPC (36 °C)[i] | 3.06 | 3.42 | N/A | 2.69 |
| 35 | quinoline | DMPC (36 °C)[i] | 1.67 | 2.79 | 2.12 | 2.44 |
| 36 | 4-chloro-3-methylphenol | DMPC (36 °C)[i] | 3.34 | 3.79 | 3.10 | 3.25 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 37 | *m*-nitroaniline | DMPC (36 °C)[i] | 2.17 | 2.54 | 2.09 | 1.50 |
| 38 | 4-*n*-pentylphenol | DMPC (36 °C)[i] | 4.31 | 5.12 | N/A | 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]ref. *4*. [e]ref. *6*. [f]ref. *11*. [e]ref. *14*. [f]ref. *15*. [g]ref. 17.

Table 2. Polyparameter model coefficients for retention behavior on two stationary phases (ref. *22*, pg 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 on hexadecane at 298 K (also known as Ostwald solubility coefficient)
[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.

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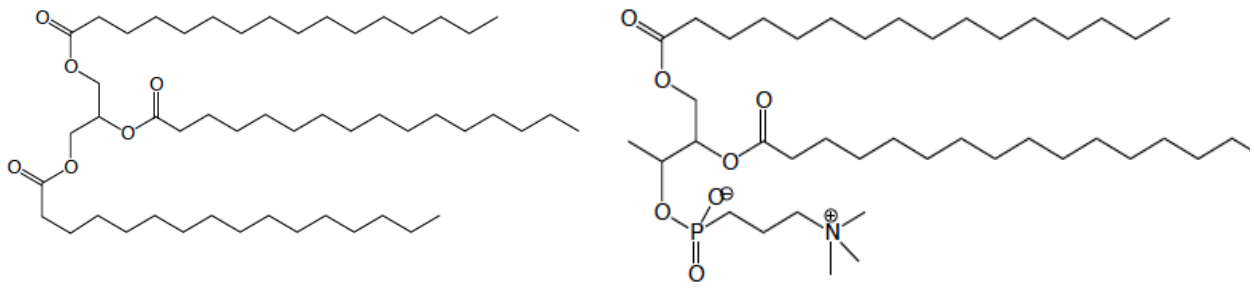

519

520 Figure 1. Examples of (left) a triglyceride storage lipid, tripalmitin ($C_{51}H_{98}O_6$, MW 807.34
521 g/mol), and (right) a membrane phospholipid, dipalmitophosphatidyl choline (DPPC,
522 $C_{40}H_{80}NO_8P$, MW 734.04 g/mol).

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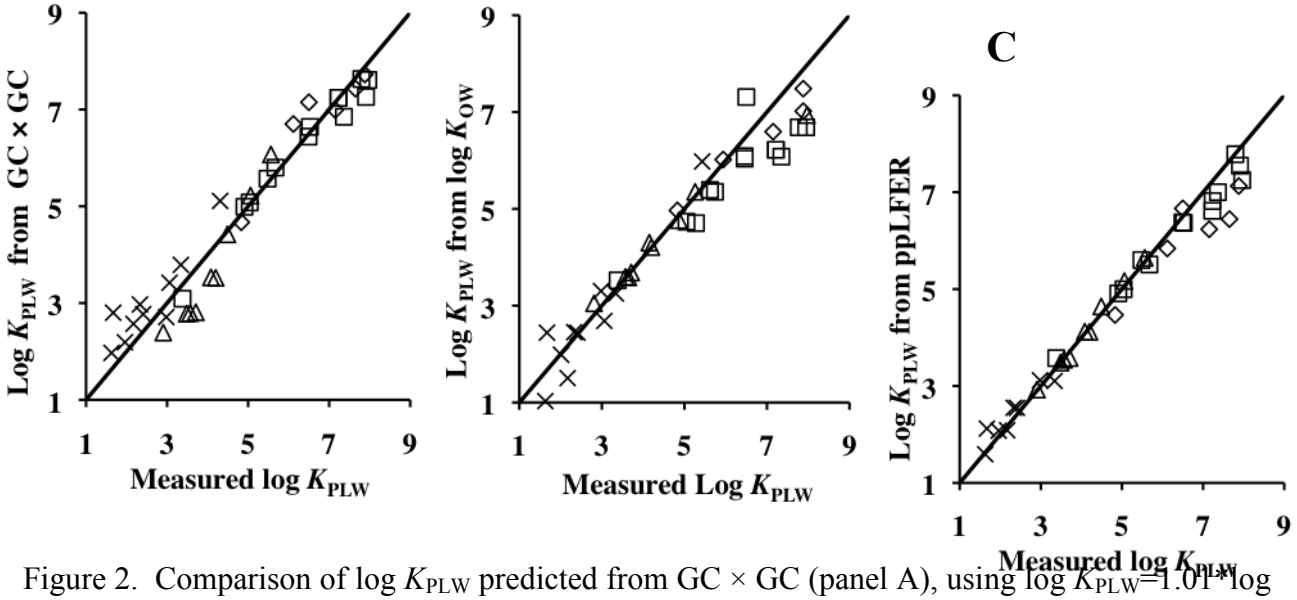


Figure 2. Comparison of log $K_{PLW}$ predicted from GC × GC (panel A), using log $K_{PLW}$=1.01*log $K_{OW}$ + 0.12 (ref. *13*, Panel B), and polyparameter model[13] (panel C). Symbols represent compound classes: PCBs (diamonds), CBs (triangles), PAHs (square), and miscellaneous (crosses). Also displayed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

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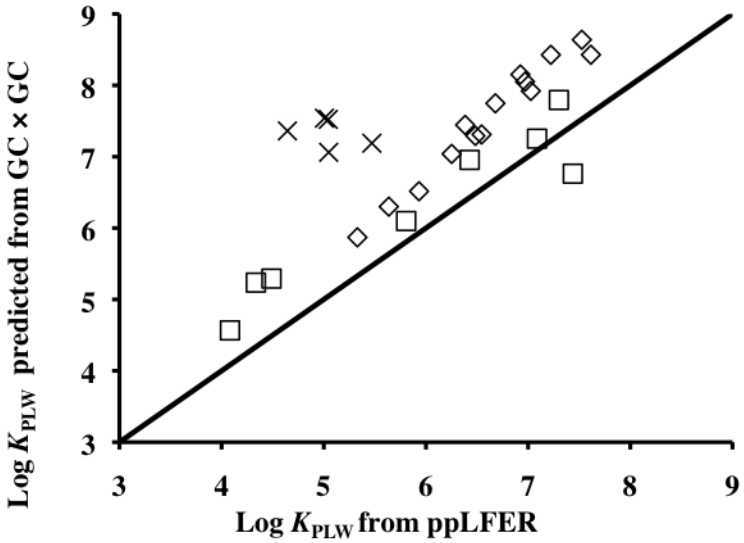


539 Figure 3. Comparison of $K_{PLW}$ predicted from GC × GC and polyparameter model[13]. Symbols represent compound classes: PCB (diamonds), OCP (squares), oxygenated-OCP (crosses). Also displayed is the 1:1 line.