

RP-HPLC measurement and quantitative structure–property relationship analysis of the *n*-octanol–water partitioning coefficients of selected metabolites of polybrominated diphenyl ethers

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Environmental context. Polybrominated diphenyl ethers (PBDEs) are ubiquitous environmental contaminants and numerous studies have demonstrated a marked increase in the levels of PBDEs in human biological tissues and fluids, especially breast milk. How PBDEs are transported through the environment, taken up by biota, transported across membranes, and metabolised depends strongly on such fundamental properties as lipophilicity ($\log K_{OW}$). However, very little data on $\log K_{OW}$ exist for PBDEs. In the present paper, the authors determine PBDE metabolites' $\log K_{OW}$ using reversed-phase high performance liquid chromatography, as recommended by the Organisation for Economic Co-operation and Development and US Environmental Protection Agency, along with quantitative structure–property relationships.

Abstract. *n*-Octanol–water partitioning coefficient ($\log K_{OW}$) values of selected hydroxylated and methoxylated polybrominated diphenyl ether metabolites were measured for the first time by reversed-phase high performance liquid chromatography (RP-HPLC) using a C18 stationary phase with a water/methanol mixture as a mobile phase. The retention parameters, $\log k_w$ (extrapolated retention indices) and k' (gradient retention indices) were calibrated to $\log K_{OW}$ by a set of calibration standards. For the PBDE metabolites investigated, extrapolated retention indices from isocratic elution seem to be more reliable and their RP-HPLC-derived $\log K_{OW}$ values were found to range from 4.63 to 7.67. Some commonly available software, including *ClogP*, *KowWin*, *AclogP*, *MlogP*, *AlogP*, *MilogP*, and *XlogP*, was used to estimate the $\log K_{OW}$ values of the analytes. Significant correlations were obtained between the RP-HPLC-derived $\log K_{OW}$ and the software-computed $\log K_{OW}$, with squared correlation coefficients (R^2) ranging from 0.793 to 0.922, but the difference between them was also significant. Then a quantitative structure–property relationship model based on topological descriptors was established and showed good reliability and predictive power for the estimation of RP-HPLC-derived $\log K_{OW}$ values of PBDE metabolites. It was applied to estimate the $\log K_{OW}$ values of some PBDE metabolites that are commercially available or have appeared in the literature. Lastly, factor analysis was carried out using the theoretical linear salvation/free-energy relationships, which indicated the average polarisability (α) and the most negative atomic partial Mulliken charge in the molecule (q^-) were the most important parameters affecting their partition between *n*-octanol and water, supporting the factorisation of $\log K_{OW}$ in bulk and electronic terms.

Additional keywords: extrapolated capacity factors, gradient elution, Kier's shape index, $\log K_{OW}$, molecular connectivity indices.

Introduction

In recent years, there has been a specific environmental focus on polybrominated diphenyl ethers (PBDEs), an important class of brominated flame retardants (BFRs) that are widely used as additives in various kinds of polymers, resins, and other substrates at concentrations ranging from 5 to 30%.^[1] They can leak out into the environment during the entire life-cycle of the products, including final waste deposition. Although their use reduces fire hazard, PBDEs also pose a risk to the environment as well as human health as they are persistent and bioaccumulative. They have already been recognised as ubiquitous environmental contaminants,^[2] and numerous studies have demonstrated a

marked increase in the levels of PBDEs in human biological tissues and fluids, especially breast milk.^[3–5]

PBDEs are structurally similar to polychlorinated biphenyls (PCBs). Therefore, their chemical properties, persistence, distribution in the environment, and metabolism in biota follow similar patterns. Hydroxylated-PBDEs (HO-PBDEs) and methoxylated-PBDEs (MeO-PBDEs) are two common classes of PBDE metabolites. Though some MeO-PBDEs found in the sea appear to be of biogenic origin,^[6] the metabolism was confirmed in biota. For example, cytochrome P450 (CYP) enzyme-mediated biotransformation of PBDEs has been shown to lead to the formation of HO-PBDEs in rodents dosed with

2,2',4,4'-tetrabromodiphenyl ether (BDE-47),^[7,8] and MeO-PBDEs can be generated via direct methoxylation of PBDEs or enzyme-mediated methylation of HO-PBDEs.^[8,9] There are already numerous studies showing that HO-PBDEs and MeO-PBDEs are present in wildlife species at high trophic levels.^[8–13]

Lipophilicity, often expressed by the *n*-octanol/water partition coefficient (log K_{OW}), is an important property that affects the environmental fate such as absorption, trans-membrane transport, bioavailability, metabolism as well as toxicity of molecules.^[14–16] Thus, the log K_{OW} value of PBDE metabolites is an important factor affecting their tissue distribution within the body. Many methods for its determination or estimation have been developed. Reversed-phase high performance liquid chromatography (RP-HPLC) is one of the commonly adopted experimental methodologies for the measurement of lipophilicity of chemical species. It is a promising method for those relatively highly lipophilic compounds (log $K_{OW} > 4$) that are difficult to determine reliably by the shake-flask method,^[17] and it has been recommended by the Organisation for Economic Co-operation and Development (OECD) and US Environmental Protection Agency (US EPA). It can be operated on both isocratic and gradient elution, whereas the latter was considered to be more suitable for highly lipophilic chemicals.^[18–20]

Quantitative structure–property relationships (QSPR) study is another widely accepted way to estimate log K_{OW} and other properties. It is based on the premise that molecular properties may be related to the chemical structures, which can be characterised by some structural descriptors. QSPR has been successfully applied in the partition property prediction of PBDEs.^[21,22] However, a training set is needed to set up the relationships between the properties and structural descriptors.

To the best of our knowledge, no experimental log K_{OW} data of HO-PBDEs and MeO-PBDEs are available in the literature. Therefore, the objective of the present paper is to measure the log K_{OW} values of selected PBDE metabolites by RP-HPLC under both isocratic and gradient elution. A series of widely applied algorithms for log K_{OW} estimation are evaluated for their ability to predict log K_{OW} values of the PBDE metabolites. Correlations between the RP-HPLC-derived and calculated log K_{OW} are analysed and discussed. A QSPR model based on topological descriptors and RP-HPLC-derived log K_{OW} is developed for the RP-HPLC-derived log K_{OW} estimation of some PBDE metabolites reported in the literature or commercially available, which have not been determined by the RP-HPLC methods presented. Further, the factor analysis is carried out by the theoretical linear salvation/free-energy relationships (TLSER) to screen the most important parameters affecting their partition between *n*-octanol and water.

Experimental

Theory of measurement of k' , k_w and K_{OW}

The RP-HPLC capacity factor, k' , of a sample species can be expressed by Eqn 1:

$$k' = \frac{t_R - t_0}{t_0} \quad (1)$$

where t_R and t_0 are the retention time of the species under investigation and the dead time, respectively. This capacity factor is related to the volume fraction of methanol (φ) in the mobile phase. Their relation can be expressed by Eqn 2:

$$\log k' = \log k_w - S\varphi \quad (2)$$

where k_w is the chromatographic hydrophobicity parameter of the species (i.e. the RP-HPLC capacity factor of the species when pure water is used as mobile phase), and S is a solute-dependent solvent strength parameter specific to the organic modifier in the stationary phase under consideration.^[19,23,24] k_w can be obtained by plotting a series of log k' values measured at various mobile phase compositions against the proportion of methanol in the mobile phase and extrapolation to 0% methanol.

The *n*-octanol–water partitioning coefficient, log K_{OW} , of the species can be correlated with its chromatographic hydrophobicity parameter, k_w by Eqn 3^[25]:

$$\log K_{OW} = a \log k_w + b \quad (3)$$

where a and b are empirical constants.

The gradient elution was also employed to describe analytes' hydrophobic indices because it can reduce retention times of highly hydrophobic compounds, and also reduce peak spreading during the elution. In this situation, the estimation relies on $k' v$. log K_{OW} regressions, and the relationship is no longer linear but can be approximated by an exponential relationship^[20]:

$$\log K_{OW} = A \exp^{Bk'} + C \quad (4)$$

where A , B and C are empirical constants.

Chemicals

2,2',4,4'-tetrabromodiphenyl ether (BDE-47) and 2,2',4,4',6-pentabromodiphenyl ether (BDE-100) were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Phenanthrene (Phen) was obtained from Sigma–Aldrich (St. Louis, MO, USA). *p,p'*-dichloro-diphenyl-trichloro-ethane (*p,p'*-DDT) was obtained from the Agro-Environmental Protection Institute of the Ministry of Agriculture, China. All other chemicals (pyrene, 2,4-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP)) were purchased from Acros (Geel, Belgium). These chemicals were used as calibration standards for log K_{OW} measurement by RP-HPLC, and their log K_{OW} were found in the literature.^[17,26–28]

PBDE metabolites used in the present study were synthesised according to the literature method^[29] and were characterised by nuclear magnetic resonance (¹HNMR), mass spectrometry (MS) and gas/liquid chromatography–mass spectrometry (GC/LC-MS). All these analytes are listed in Table 1. Methanol was of HPLC grade and was obtained from Tedia (Fairfield, OH, USA).

RP-HPLC conditions

An Agilent 1200 Series HPLC (Palo Alto, CA, USA) with a quaternary pump, vacuum degasser, autosampler, and a controlled thermostat was used in all experiments. Analytes were detected with an Agilent 1200 Series diode array detector set at 254 and 210 nm and a bandwidth of 4 nm. Measurements were made at 30°C, on an Agilent Rx-C18 column (5 μm, 250 × 4.6 mm inner diameter, i.d.), a column made by chemically bonding a monolayer of dimethyl-octadecyl silane stationary phase to a porous-silica microsphere with a controlled pore size of 80 Å.

Mobile phases for isocratic elution ranged from 75 to 95% (75, 80, 85, 90, and 95%, respectively) methanol with 0.2% (v/v) acetic acid in water. The pH values of different composition mobile phases ranged from 4.2 to 4.9. A flow rate of 1.0 mL min⁻¹ was adopted. Sodium nitrate was used as an unretained compound to determine the dead time (t_0). For gradient elution, methanol content of the mobile phase was

Table 1. Capacity factors and log K_{ow} of calibration chemicals and polybrominated diphenyl ether (PBDE) metabolites
 p,p' -DDT, p,p' -dichloro-diphenyl-trichloro-ethane, BDE, brominated diphenyl ether, and S , solute-dependent solvent strength parameter in Eqn 2

No.	Compounds	Literature log K_{ow}	Extrapolated retention indices					Gradient retention indices				
			log k_w	S	R^2	Estimated log K_{ow}	Residual	k'	Estimated log K_{ow}	Residual		
SD1	2,4-dichlorophenol	3.21 ^[26]	2.05 (±0.07)	2.62 (±0.08)	0.9971	3.10	-0.11	18.38	3.27	0.06		
SD2	2,4,6-trichlorophenol	3.75 ^[26]	2.75 (±0.05)	3.18 (±0.06)	0.9988	3.75	0.00	23.01	3.64	-0.09		
SD3	Phenanthrene	4.5 ^[17]	3.82 (±0.05)	3.88 (±0.06)	0.9994	4.74	0.24	30.23	4.90	0.40		
SD4	Pentachlorophenol	5.04 ^[26]	4.10 (±0.03)	4.26 (±0.04)	0.9997	5.00	-0.04	30.11	4.87	-0.17		
SD5	Pyrene	5.2 ^[28]	4.28 (±0.05)	4.14 (±0.04)	0.9994	5.17	-0.03	32.99	5.79	0.59		
SD6	BDE-47	6.81 ^[27]	6.00 (±0.03)	5.94 (±0.04)	0.9999	6.76	-0.05	35.38	6.86	0.05		
SD7	p,p' -DDT	6.5 ^[17]	5.87 (±0.06)	6.07 (±0.07)	0.9996	6.64	0.14	33.60	6.03	-0.43		
SD8	BDE-100	7.24 ^[27]	6.41 (±0.02)	6.23 (±0.03)	1.000	7.14	-0.10	36.59	7.53	0.29		
1	2'-Hydroxy-2,4-diBDE		3.70 (±0.07)	4.18 (±0.08)	0.9989	4.63		27.13	4.22			
2	3'-Hydroxy-2,4-diBDE		3.81 (±0.07)	4.25 (±0.08)	0.9989	4.73		27.70	4.33			
3	6'-Chloro-2'-hydroxy-2,4-diBDE		4.19 (±0.07)	4.65 (±0.08)	0.9990	5.08		28.69	4.53			
4A	2'-Hydroxy-2,4,4'-triBDE		4.65 (±0.08)	5.01 (±0.10)	0.9988	5.50		30.23	4.91			
5A	6'-Hydroxy-2,2',4'-triBDE		4.29 (±0.07)	4.73 (±0.08)	0.9992	5.18		28.96	4.59			
6A	4'-Hydroxy-2,2',4'-triBDE		4.52 (±0.06)	4.86 (±0.07)	0.9994	5.39		30.00	4.84			
7A	2'-Hydroxy-2,3',4,5'-tetraBDE		5.36 (±0.04)	5.57 (±0.05)	0.9998	6.17		32.58	5.64			
8A	6-Hydroxy-2,2',4,4'-tetraBDE		5.82 (±0.03)	5.97 (±0.04)	0.9999	6.59		33.62	6.04			
10A	4'-Hydroxy-2,2',4,5'-tetraBDE		5.28 (±0.04)	5.48 (±0.05)	0.9998	6.09		32.42	5.58			
11A	6'-Chloro-2'-hydroxy-2,3',4,5'-tetraBDE		5.41 (±0.05)	5.63 (±0.06)	0.9997	6.22		32.60	5.65			
12A	5-Chloro-6-hydroxy-2,2',4,4'-tetraBDE		5.77 (±0.04)	5.92 (±0.04)	0.9999	6.54		33.42	5.96			
13A	6-Hydroxy-2,2',3,4,4'-pentaBDE		5.83 (±0.02)	5.97 (±0.02)	1.000	6.61		33.68	6.07			
14A	2-Hydroxy-2,2',3,4,5-pentaBDE		5.82 (±0.03)	5.97 (±0.04)	0.9999	6.60		33.40	5.95			
15A	2-Hydroxy-2',3,4,4',5-pentaBDE		5.82 (±0.03)	5.96 (±0.04)	0.9999	6.60		33.42	5.96			
16A	4-Hydroxy-2,2',3,4',5-pentaBDE		5.72 (±0.03)	5.84 (±0.04)	0.9999	6.50		33.63	6.04			
17A	6-Hydroxy-2,2',3,4,4',5-hexaBDE		6.45 (±0.03)	6.49 (±0.03)	0.9999	7.17		34.75	6.54			
18A	3-Hydroxy-2,2',4,4,6'-pentaBDE		5.64 (±0.04)	5.81 (±0.04)	0.9998	6.43		33.33	5.92			
19A	2'-Hydroxy-2,3',4,4'-tetraBDE		5.02 (±0.06)	5.35 (±0.07)	0.9995	5.85		31.28	5.21			
20A	2'-Hydroxy-2,4,4'-triBDE		4.25 (±0.06)	4.68 (±0.07)	0.9992	5.13		28.87	4.57			
4B	2'-Methoxy-2,4,4'-triBDE		5.25 (±0.06)	5.39 (±0.07)	0.9995	6.06		32.71	5.69			
5B	6'-Methoxy-2,2',4'-triBDE		4.90 (±0.05)	5.13 (±0.06)	0.9996	5.74		31.51	5.28			
6B	4'-Methoxy-2,2',4'-tetraBDE		5.34 (±0.04)	5.44 (±0.05)	0.9998	6.15		33.20	5.87			
7B	2'-Methoxy-2,3',4,5'-tetraBDE		6.16 (±0.02)	6.01 (±0.03)	1.000	6.91		35.84	7.10			
8B	6-Methoxy-2,2',4,4'-tetraBDE		6.44 (±0.01)	6.32 (±0.01)	1.000	7.17		36.00	7.19			
9B	5-Methoxy-2,2',4,4'-tetraBDE		5.96 (±0.03)	5.98 (±0.04)	0.9999	6.72		34.53	6.44			
10B	4'-Methoxy-2,2',4,5'-tetraBDE		5.91 (±0.03)	5.91 (±0.04)	0.9999	6.68		34.55	6.45			
11B	6'-Chloro-2'-methoxy-2,3',4,5'-tetraBDE		6.61 (±0.03)	6.43 (±0.04)	0.9999	7.33		36.37	7.41			
12B	5-Chloro-6-methoxy-2,2',4,4'-tetraBDE		6.62 (±0.03)	6.44 (±0.03)	1.000	7.34		36.35	7.39			
13B	6-Methoxy-2,2',3,4,4'-pentaBDE		6.26 (±0.01)	6.24 (±0.01)	1.000	7.00		6.71	6.71			
14B	6-Methoxy-2,2',3,4',5-pentaBDE		6.65 (±0.03)	6.47 (±0.03)	1.000	7.36		7.35	7.35			
15B	2-Methoxy-2',3,4,4',5-pentaBDE		6.62 (±0.01)	6.40 (±0.01)	1.000	7.34		36.49	7.48			
16B	4-Methoxy-2,2',3,4',5-pentaBDE		6.42 (±0.01)	6.27 (±0.02)	1.000	7.15		36.15	7.28			
17B	6-Methoxy-2,2',3,4,4',5-hexaBDE		6.98 (±0.03)	6.84 (±0.04)	0.9999	7.67		37.23	7.94			
18B	3-Methoxy-2,2',4,4,6'-pentaBDE		6.35 (±0.01)	6.25 (±0.01)	1.000	7.08		35.79	7.07			

increased linearly from 20 to 100% over 60 min at a flow rate of 1.3 mL min^{-1} . Dead time (t_0) was determined under isocratic elution with water/methanol at a volume ratio of 80 : 20 using sodium nitrate as the unretained compound.^[19]

In all chromatographic runs, samples were dissolved in methanol and concentrations generally ranged from 100 to $500 \mu\text{g mL}^{-1}$. All the analytes were injected as single standards and the injection volume was $10 \mu\text{L}$.

Estimation of log K_{OW}

log K_{OW} of the selected HO-PBDE and MeO-PBDE metabolites were estimated using a series of widely adopted algorithms, including *ClogP*, *KowWin*, *AclogP*, *MlogP*, *AlogP*, *MilogP* and *XlogP*. *AlogP* is based on E-state indices (neural network), *MilogP* is based on counting atoms, bonds, fragments or functional groups, *XlogP* is based on atom contributions with correction factors, and the remaining algorithms are fragment-based. *ClogP* was implemented in *ChemBioOffice Ultra 2008* Trial Version (Cambridge Software Co., Cambridge, MA, USA); all the others were obtained from the internet calculator: <http://www.vcclab.org/lab/alogs/> (accessed 6 October 2008).

Calculation of structural descriptors and QSPR analysis

Molecular geometry was optimised and quantum chemical descriptors were calculated by the semi-empirical orbital *MOPAC 7.0* procedures according to method AM1. The calculated individual structural descriptors were the average polarisability (α), dipole moment (μ), energy of the highest occupied molecular orbital (Ehomo), energy of the lowest unoccupied molecular orbital (Elumo), the most positive partial Mulliken charge on a hydrogen atom ($q\text{H}^+$), and the most negative atomic partial Mulliken charge in the molecule (q^-). Molecular volume (V_m) was calculated using the *HyperChem package 7.5* (Evaluation Version) (Hyper-Cube, Waterloo, Canada). Molecular connectivity indices and Kier's molecular shape index were calculated according to their definitions by Matlab 7.0.

Statistical analysis and model validation

All the regressions were performed on *SPSS 11.5* (SPSS Inc., Chicago, IL, USA). The goodness of the correlation was tested by the correlation coefficient (R^2), the F-test (F), and standard error of estimation (s.e.).

Cross-validation is a practical and reliable method for testing the significance of a model. Hence, the leave-one-out (LOO) method was used to validate the final model. In LOO cross-validation of the current work, in the first step one of the target compounds was excluded from the training set, and a model was built for $n - 1$ samples between descriptors and log K_{OW} using multilinear regression. Next, the value of log K_{OW} was predicted from the model for the excluded compound. The procedure above was repeated until every sample in the total data set was used for a prediction. The predictive ability of the models is characterised by the cross-validated correlation coefficient (R_{cv}^2) given by:

$$R_{cv}^2 = 1 - \frac{\sum_{i=1}^n (Y_i - \hat{Y}_i)^2}{\sum_{i=1}^n (Y_i - \bar{Y})^2} \quad (5)$$

where Y_i , \hat{Y}_i and \bar{Y} are the experimental, predicted and average (over the entire data set) values of the dependent variable respectively.

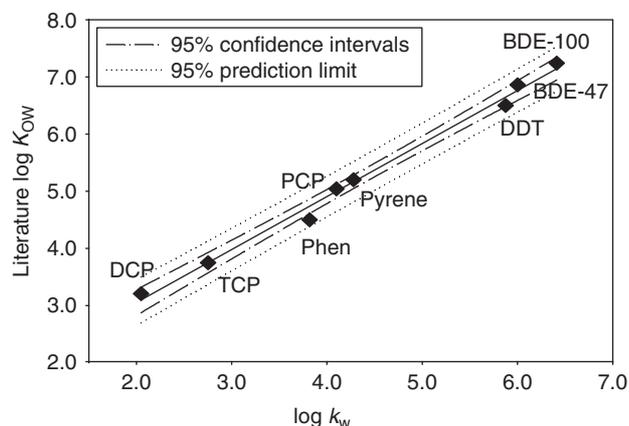


Fig. 1. Correlation between literature log K_{OW} and log k_w , the extrapolated retention indices of calibration chemicals ($\log K_{OW} = 0.92 \log k_w + 1.20$, $R^2 = 0.993$, $R_{cv}^2 = 0.987$, s.e. = 0.108, $F = 936.4$). See text for full names of compounds.

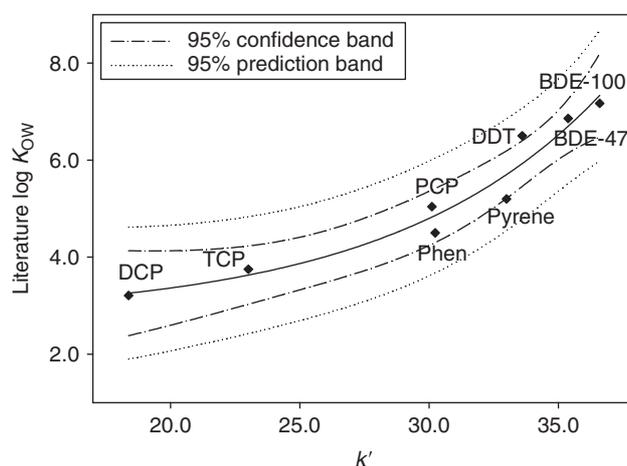


Fig. 2. Correlation between literature log K_{OW} and k' , gradient retention indices of calibration chemicals ($\log K_{OW} = 0.0440 \exp(0.128k') + 2.809$, $R^2 = 0.947$, $R_{cv}^2 = 0.895$, s.e. = 0.40, $F = 44.7$). See text for full names of compounds.

Results and discussion

Measured log K_{OW} by RP-HPLC

Under isocratic elution, linear relationships ($R^2 > 0.99$) between log k' and φ were observed for all the calibration standards and PBDE metabolites. All the log k_w , extrapolated retention indices, and S values obtained are tabulated in Table 1. A plot of log k_w v. S of PBDE metabolites revealed a straight line conforming to Eqn 6, and the good linearity indicated a uniform retention mechanism.^[30]

$$S = 0.77 (\pm 1.3 \times 10^{-2}) \log k_w + 1.40 (\pm 7.5 \times 10^{-2}) \quad (6)$$

where $n = 34$, $R^2 = 0.9905$, s.e. = 0.0668, and $F = 3401.5$.

Figs 1 and 2 show the calibration curves correlating the values of log k_w (extrapolated retention indices) and k' (gradient retention indices) of the calibration standards to their literature log K_{OW} values. A linear correlation was observed between log k_w and log K_{OW} , whereas an exponential relationship was obtained between log K_{OW} and k' .

The $\log K_{OW}$ values of the PBDE metabolites obtained from their measured capacity factors are listed in Table 1. The two sets of estimated $\log K_{OW}$ values obtained by extrapolated retention indices ($\log K_{OW(Extra)}$) and gradient retention indices ($\log K_{OW(Gra)}$) bear a statistically significant correlation (Eqn 7):

$$\log K_{OW(Gra)} = 1.5523 \exp(0.2107 \log K_{OW(Extra)})$$

$$(n = 34, R^2 = 0.9744) \quad (7)$$

When $\log K_{OW(Extra)} < 7$, $\log K_{OW}$ estimated by extrapolated retention indices were slightly higher than those obtained from gradient elution, and the difference generally narrowed as the hydrophobicity of the compounds increased; in other cases, $\log K_{OW}$ estimated by gradient elution were slightly higher than those obtained from extrapolated retention indices (Fig. A1 of the Accessory publication). This is partly consistent with the phenomena observed in the literature.^[31,32] The high organic modifier fraction required for the highly hydrophobic compounds in the isocratic elution may make the similarity between the stationary phase–eluent system and the octanol–water system become increasingly tenuous, and gradient elution is considered to be more suitable for the highly hydrophobic compounds,^[18,20] but linear correlation between $\log k_w$ and $\log K_{OW}$ has been observed in the literature for $\log K_{OW}$ as high as 8.^[32] Judging from the significant similarity of these two calibration curves between $\log k_w$ and $\log K_{OW}$ for $\log K_{OW} \leq 7.2$ of calibration chemicals in the present study, the $\log K_{OW}$ values of the PBDE metabolites estimated by extrapolated retention indices appears to be more reliable, based on the stronger statistical correlation of that regression.

However, previous studies found that gradient elution gave better estimates of $\log K_{OW}$ values.^[18–20] This contradicted findings were due to the methods utilised, including initial composition of the mobile phase, the increased rate and mode (linear or non-linear) of methanol content, and the flow rate. The set of reference compounds and their uncertain $\log K_{OW}$ values may have had an influence as well.

QSPR analysis

Computer-assisted calculation is a useful tool to estimate $\log K_{OW}$ values of chemicals when no experimental values are available. Table A1 of the Accessory publication lists all the $\log K_{OW}$ values of the PBDE metabolites calculated by some popular methods. Table 2 in the present paper summarises the correlations between the RP-HPLC-measured $\log K_{OW}$ and those obtained by computation. It can be seen that $\log K_{OW}$ estimated by the computation software correlates significantly with the RP-HPLC-measured values ($\alpha = 0.05$); *ClogP* is considered as the reference algorithm for $\log K_{OW}$ calculation taking R^2 , a and b into account (Table 2).

Fig. 3 shows the plot of the RP-HPLC-measured $\log K_{OW}$ against the estimated $\log K_{OW}$ values by *ClogP* and the method with the highest R^2 , *MlogP*, both using fragment-based algorithms. Obviously both methods describe the same trend revealed by RP-HPLC measurements; *ClogP* simulated the $\log K_{OW}$ values very well but overestimated by ~ 0.4 log units generally, whereas *MlogP* underestimated and the difference grew larger as the hydrophobicity of the compounds increased.

The deviations between universal calculated and RP-HPLC-derived $\log K_{OW}$ are significant, which may be attributed to the deficient $\log K_{OW}$ database for fragment generation. It seems necessary to establish models suited to PBDE metabolites with

Table 2. Linear relationships between reversed-phase high performance liquid chromatography (RP-HPLC)-measured and calculated $\log K_{OW}$ of selected polybrominated diphenyl ether (PBDE) metabolites

$$(\log K_{OW})_{Cal} = a + b \times (\log K_{OW})_{RP-HPLC}$$

	a	b	R^2	s.e.	F	n
<i>AlogP</i>	2.24	0.62	0.851	0.22	182.8	34
<i>AclogP</i>	0.68	0.85	0.833	0.31	159.8	34
<i>MilogP</i>	0.24	1.00	0.845	0.36	175.0	34
<i>KowWin</i>	-0.94	1.19	0.904	0.32	301.5	34
<i>XlogP</i>	0.56	0.93	0.793	0.39	122.6	34
<i>ClogP</i>	0.47	1.00	0.896	0.28	276.5	34
<i>MlogP</i>	1.55	0.498	0.922	0.12	377.2	34

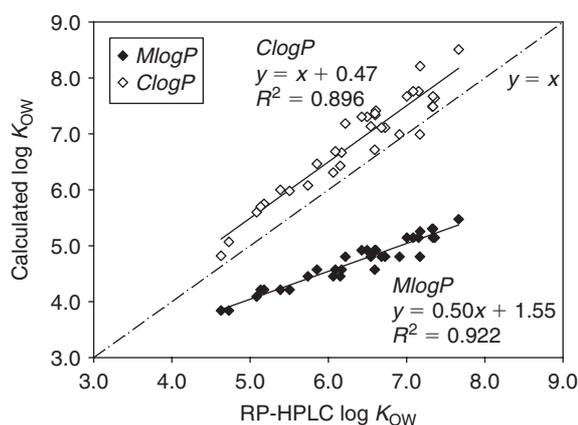


Fig. 3. Correlations between reversed-phase high performance liquid chromatography (RP-HPLC) $\log K_{OW}$ values and calculated $\log K_{OW}$ values of selected polybrominated diphenyl ether (PBDE) metabolites.

high accuracy based on the specific PBDE metabolites' RP-HPLC-derived $\log K_{OW}$. In the current work, molecular topological descriptors, including molecular connectivity indices, which have proved to be particularly successful in estimating various partitioning properties of persistent organic pollutants,^[33] and Kier's shape index (Kappa index), which can reflect the flexibility attributes of structures,^[34] were used to set up the QSPR.

The resulting model, Eqn 8, is given by stepwise regression analysis of $\log K_{OW}$ of the compounds v. these molecular topological descriptors.

$$\log K_{OW} = -3.05 + 1.60 {}^3K + 1.66 \times 10^{-3} \times {}^7\chi \quad (8)$$

where $n = 34$, $R^2 = 0.984$, $R_{cv}^2 = 0.981$, s.e. = 0.107, and $F = 936.4$.

It is evident that the model is able to correlate $\log K_{OW}$ to the third-order Kier's shape index (3K) and the seventh-order molecular connectivity index (${}^7\chi$) with high statistical significance ($R^2 = 0.984$ and $R_{cv}^2 = 0.981$). Results also showed ${}^7\chi$ explained 86.0% of the total variance according to the analysis of variance. Both ${}^7\chi$ and 3K are related to the molecular volume (V_m) (Eqns 9–10); 3K also can reflect the relative positions of the HO (or MeO) group and the bromine substituents on the PBDE metabolites. For example, the 3K values of the

isomeric 6-methoxy-2,2',4,4'-tetraBDE (8B) and 5-methoxy-2,2',4,4'-tetraBDE (9B) are 4.1401 and 3.8925, respectively.

$${}^7\chi = 3.25V_m - 609.79 \quad (R^2 = 0.8721, n = 34) \quad (9)$$

$${}^3K = 4.1 \times 10^{-3}V_m + 0.14 \quad (R^2 = 0.8058, n = 34) \quad (10)$$

In order to assess the reliability and external predictive power of the model (Eqn 8) developed in the present study, the whole data set was divided into the test set and the training set, and LOO cross-validation for the training set was performed. The test set contained nine PBDE metabolites (2, 5A, 19A, 6B, 12A, 13A, 13B, 11B, 14B). Results show the predicted log K_{OW} values are consistent with the experimental values (R^2 for the training set and the test set were 0.983 and 0.988 respectively), which confirmed the good reliability and predictive power of the QSPR model. Fig. 4 shows the plot of the predicted v. experimental

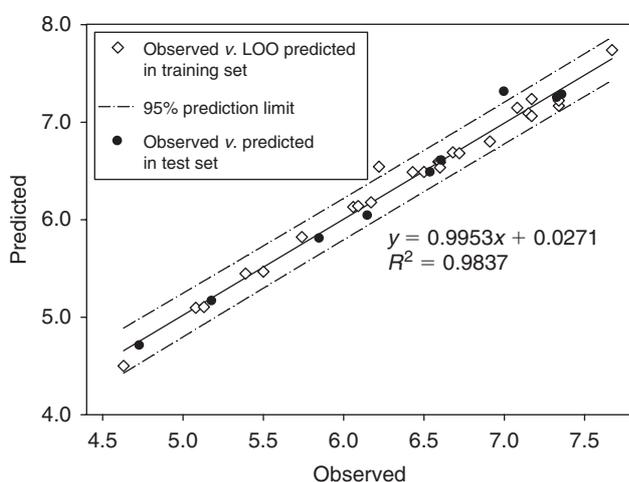


Fig. 4. Correlations between predicted and observed log K_{OW} in training set and test set. LOO, leave-one-out.

log K_{OW} for the training set and the test set. Finally, the QSPR model was used to estimate the log K_{OW} values of some of the PBDE metabolites that are commercially available or have appeared in the literature. Results are tabulated in Table 3.

MTLSER analysis

Topological descriptors are considered to be the most efficient in describing and quantifying properties related to non-specific molecular interactions such as hydrophobic and dispersion forces.^[33] However, they are not so good at interpreting physical meaning, but linear solvation/free-energy relationships (LSERs) are.^[35,36] In order to get some insightful information in the present study, a modified theoretical LSER (MTLSER) model,^[37] as shown in Eqn 11, was employed because there is no experimental solvatochromic parameter available for PBDE metabolites.

$$XYZ = XYZ_0 + m\alpha + s\mu + a_1E_{lumo} + a_2qH^+ + b_1E_{homo} + b_2q^- \quad (11)$$

where XYZ represents solubility or solvent-dependent properties (often expressed as the logarithm of measured properties), and α , μ , E_{homo} , E_{lumo} , qH^+ and q^- represent average polarisability, dipole moment, energy of the highest occupied molecular orbital, energy of the lowest unoccupied molecular orbital, the most positive partial Mulliken charge on a hydrogen atom, and the most negative atomic partial Mulliken charge in the molecule, respectively, with m , s , a_i , b_i ($i = 1, 2$) being empirical constants.

For all the log K_{OW} data analysed, the step-wise regression result was expressed as Eqn 12:

$$\log K_{OW} = -1.13 + 0.0526\alpha + 4.59q^- \quad (12)$$

where $n = 34$, $R^2 = 0.930$, $R_{cv}^2 = 0.916$, s.e. = 0.22, and $F = 205.6$.

Table 3. Predicted reversed-phase high performance liquid chromatography (RP-HPLC)-derived log K_{OW} values of some metabolites of polybrominated diphenyl ethers (PBDEs) BDE, brominated diphenyl ether

Compounds	log K_{OW}		Compounds	log K_{OW}	
	Eqn 8	Eqn 12		Eqn 8	Eqn 12
2'-Hydroxy-4-diBDE	3.81	4.51	2'-Methoxy-2,4-diBDE	5.29	4.68
3'-Hydroxy-2,4,4'-triBDE	5.32	5.70	5-Hydroxy-2,2',4,4'-tetraBDE	6.15	6.20
4'-Hydroxy-2,4,6-triBDE	5.84	5.49	2'-Methoxy-4-diBDE	4.45	4.91
4-Hydroxy-2,2',3,4'-tetraBDE	5.81	6.05	3'-Methoxy-2,4,4'-triBDE	5.93	6.41
3-Hydroxy-2,2',4,4'-tetraBDE	5.72	6.20	4-Methoxy-2,2',3,4'-tetraBDE	6.35	6.35
5-Hydroxy-2,2',4,4'-tetraBDE	6.10	6.04	3-Methoxy-2,2',4,4'-tetraBDE	6.30	6.68
4'-Hydroxy-2,3',4,6-tetraBDE	6.48	5.93	5-Methoxy-2,2',4,4'-tetraBDE	6.68	6.74
6'-Hydroxy-2,2',4,5'-tetraBDE	5.86	6.15	6'-Methoxy-2,2',4,5'-tetraBDE	6.48	6.62
3'-Chloro-6'-hydroxy-2,2',4,5'-tetraBDE	6.55	6.49	6-Methoxy-2,2',3,4,5'-tetraBDE	7.29	7.24
6-Hydroxy-2,2',3,4,5'-tetraBDE	6.61	6.67	6-Methoxy-2,2',3,3',4-pentaBDE	6.98	7.37
6-Hydroxy-2,2',3,3',4-pentaBDE	6.30	6.43	6'-Methoxy-2,2',4,4',5-pentaBDE	7.50	7.27
6'-Hydroxy-2,2',4,4',5-pentaBDE	6.83	6.82	5'-Methoxy-2,2',4,4',5-pentaBDE	7.35	7.27
5'-Hydroxy-2,2',4,4',5-pentaBDE	6.71	6.67	6-Methoxy-2,2',4,4',5-pentaBDE	7.22	7.16
6-Hydroxy-2,2',4,4',5-pentaBDE	6.55	6.78	6-Methoxy-2,2',3,4,4',6-hexaBDE	8.01	7.86
4'-Hydroxy-2,3',4,5',6-pentaBDE	7.11	6.53	3'-Methoxy-2,2',4,4',5,6'-hexaBDE	7.79	7.81
6-Hydroxy-2,2',3,4,4',6-hexaBDE	7.34	7.11	6-Methoxy-2,3,3',4,4',5-hexaBDE	7.84	7.87
3'-Hydroxy-2,2',4,4',5,6'-hexaBDE	7.16	6.91	3'-Methoxy-2,4-diBDE	5.29	5.95
6-Hydroxy-2,3,3',4,4',5-hexaBDE	7.18	7.29			

It seems that the polarisability (α) and q^- of the analytes are the two most significant parameters affecting PBDE metabolite log K_{OW} . It is consistent with the concept that lipophilicity is the outcome of bulk (expressed by α here) and polar factors (expressed by q^- here).^[38] Greater molecular size resulted in higher log K_{OW} , and increasing absolute values of q^- led to a decrease in log K_{OW} values because q^- characterises the molecular ability to donate electrons or accept protons in intermolecular electrostatic interaction and PBDE metabolites with large absolute values of q^- tend to have great intermolecular electrostatic interaction. It is also quite similar to the phenomena observed by Wang et al.^[21] in their study of PBDE partition between *n*-octanol and air (K_{OA}) where q^- , μ and α were found to be the most significant parameters.

Eqn 12 was also used to predict these PBDE metabolite log K_{OW} values, which are listed in Table 3. Compared with the values calculated by Eqn 8, most agreed very well, with deviations lower than 0.49,^[38] except four cases, with the highest deviation at 0.70. However, Eqn 12-derived log K_{OW} values are conformation-dependent; the deviations may be reduced by sufficient optimisation of the geometries of all analytes.

Conclusions

In the present study, both isocratic elution and gradient elution RP-HPLC have been used to measure the *n*-octanol–water partitioning coefficients of selected hydroxylated and methoxylated metabolites of polybrominated diphenyl ethers. To the best of our knowledge, this is the first time that K_{OW} of PBDE metabolites are experimentally measured. Based on the stronger statistical correlation of that regression between the extrapolated log k_w and log K_{OW} of the calibration standards up to 7.2, the extrapolated retention index-derived log K_{OW} values appeared to be more reliable for the PBDE metabolites. These RP-HPLC-measured log K_{OW} values were compared with those obtained by computation using various available software, and a fragment-based algorithm was found to produce estimated values closest to the measured log K_{OW} . Then a QSPR model of good reliability and predictive power for the estimation of RP-HPLC-derived log K_{OW} of PBDE metabolites was established and applied. Theoretical linear salvation/free-energy relationship analysis showed that the two most important parameters affecting PBDE metabolites' partition between water and *n*-octanol are polarisability and the most negative atomic partial Mulliken charge in the molecule (q^-), which confirmed the concept that lipophilicity can be factorised in bulk and polarity terms.

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