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Hydrophobicity-dependent QSARs to predict the toxicity of perfluorinated carboxylic acids and their mixtures

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ABSTRACT

Perfluorinated carboxylic acids (PFCAs) have wide industrial applications because of their unique physicochemical characteristics. However, data on the toxicity of much of this chemical class is lacking, particularly with regard to mixture toxicity. In this study, the toxicity of individual PFCAs and their mixtures to *Photobacterium phosphoreum* were observed. There was a tendency of increasing toxicity from C3 to C14 PFCA and a tendency of decreasing toxicity from C14 to C18 PFCA because of "the maximum tolerance of the cell membrane". Using the equivalent $\log K_{OW}$ (octanol–water partition coefficient) and $\log K_{SD}$ (C_{18}-Empore™ disks/water partition coefficient), two linear quantitative structure–activity relationship (QSAR) models were formulated. This indicated both K_{SD} and K_{OW} can describe the hydrophobicity of a single chemical. However, for the PFCA mixtures, K_{MD} is the more reasonable parameter than K_{owmix} to describe the hydrophobicity because only the equivalent $\log K_{MD}$ could be used to predict the mixture toxicity.

1. Introduction

It has been widely accepted that toxic effects are usually generated by multiple mixtures rather than single chemicals in the environment. However, chemical risk assessment is always based on the effects of single substances because there are few data regarding mixture toxicity. Therefore, research on the mixture toxicity of chemicals is needed and will improve health and environmental risk assessment.

Perfluorinated carboxylic acids (PFCAs) have wide industrial applications because of their unique physicochemical characteristics, such as chemical and thermal inertness, low surface energy and high surface-active properties (Key et al., 1997). Because of these properties, PFCAs have great stability and persistency in the environment and therefore, are a ubiquitous presence in environmental media. Moreover, PFCAs can induce a proliferation of peroxisomes and cause alterations in lipid metabolism in rodent livers. Therefore, some studies have focused on the toxicity of PFCAs; these studies have been conducted in human colon carcinoma (HCT 116) cells (Kleszczynski et al., 2007), a promyelocytic leukemia rat cell line (IPC-81), the rat glioma cell line (C6), *Vibrio fischeri*, *Chlorella vulgaris*, the diatom *Skeletonema marinoi* and the blue-green alga *Geitlerinema amphibium* (Latala et al., 2009). However, there are currently no data on mixture toxicity of PFCAs, and no method for prediction of the mixture toxicity of PFCAs currently exists.

Abbreviations: $\log K'_{OW}$, equivalent $\log K_{OW}$; $\log K'_{SD}$, equivalent $\log K_{SD}$; $\log K'_{MD}$, equivalent $\log K_{MD}$.

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Table 1 – Toxicity experiment results and the chemical descriptors of single PFCAs.

	Molecular formula	Molecular diameter (nm)	$\log K_{OW}$	$\log K_{SD}$	$\log(1/EC_{50})$ (mol L^{-1})		
					Obs.	Pre.	Diff.
C3 PFCA							
Pentafluoropropionic acid	$C_3HF_5O_2$	0.4	1.47	2.16	4.13	4.15	−0.03
C4 PFCA							
Perfluorobutyric acid	$C_4HF_7O_2$	0.5	2.43	3.12	4.18	4.15	0.03
C5 PFCA							
Perfluoropentanoic acid	$C_5HF_9O_2$	0.6	3.40	4.08	4.21	4.18	0.03
C6 PFCA							
Perfluorohexanoic acid	$C_6HF_{11}O_2$	0.7	4.37	5.05	4.26	4.25	0.01
C7 PFCA							
Perfluoroheptanoic acid	$C_7HF_{13}O_2$	0.8	5.33	6.00	4.30	4.35	−0.05
C8 PFCA							
Pentadecafluorooctanoic acid	$C_8HF_{15}O_2$	0.9	6.30	6.97	4.45	4.46	−0.01
C9 PFCA							
Perfluorononanoic acid	$C_9HF_{17}O_2$	1.0	7.27	7.93	4.60	4.58	0.02
C10 PFCA							
Perfluorodecanoic acid	$C_{10}HF_{19}O_2$	1.1	8.23	8.89	4.70	4.71	−0.01
C12 PFCA							
Perfluorododecanoic acid	$C_{12}HF_{23}O_2$	1.3	10.16	10.81	4.92	4.94	−0.02
C14 PFCA							
Perfluorotetradecanoic acid	$C_{14}HF_{27}O_2$	1.5	12.10 14.03[a]	12.74 14.66[a]	5.16	5.08	0.08
C16 PFCA							
Perfluorohexadecanoic acid	$C_{16}HF_{31}O_2$	1.7	11.86[b] 15.96[a]	12.50[b] 16.58[a]	5.00	5.07	−0.07
C18 PFCA							
Perfluorooctadecanoic acid	$C_{18}HF_{35}O_2$	1.9	9.23[b]	9.88[b]	4.86	4.84	0.02

[a] Kowwin v1.67 from EPI database.
[b] The equivalent $\log K_{OW}$.

There is evidence that the hydrophobicity-dependent QSAR approach can be used to predict the toxicity of single PFCAs. Viability tests were performed on C6–C18 PFCAs with human colon carcinoma (HCT 116) cells, and the results showed that EC_{50} values decreased with elongation of the fluorocarbon chain (C14 > C12 > C10 > C9 > C8 > C7 > C6) (Kleszczynski et al., 2007). Furthermore, similar relationships between the toxicity of PFCAs and the perfluorocarbon chain length were also found in previous studies (Latala et al., 2009; Mulkiewicz et al., 2007). Based on the available toxicity data, it seems that there is a linear relationship between hydrophobicity and the toxicity of single PFACs. If this relationship can be demonstrated definitively, it may provide the foundation for a hydrophobicity-dependent QSAR to predict the toxicity of PFCAs.

There are many physicochemical parameters that have been proposed to describe the hydrophobicity of single chemicals, but only two of them have been extended to the assessment of mixtures. One is the octanol/water partition coefficient (K_{OW}), which was extended to the assessment of mixtures as follows (Altenburger et al., 2003; Roberts, 1991):

$$\log K_{owmix} = \log\left[\sum x_i (K_{OW})_i\right] \quad (1)$$

where K_{owmix} is the K_{OW} of the mixture, x_i is the molar fractions of the components and their K_{OW} value is $(K_{OW})_i$.

The other is the C_{18}-Empore™ disks/water partition coefficient (K_{SD}), which was found to have the following relationship with $\log K_{OW}$ (Verhaar et al., 1995):

$$\log K_{SD} = 0.995 \log K_{OW} + 0.70 \quad (2)$$

$n = 18$; $R^2 = 0.93$; SE = 0.24.

Furthermore, it was extended for use in the study of mixtures by using Eq. (3) as follows:

$$K_{MD} = \frac{W}{V} \times \frac{\sum_{i=1}^{n}(Q^0_{water,i}/1 + (W/VK_{SDi}))}{\sum_{i=1}^{n}(Q^0_{water,i}\sum_{i=1}^{n}(Q^0_{water,i}/1 + (W/VK_{SDi}))} \quad (3)$$

where K_{MD} is the C_{18}-Empore™ disk/water partition coefficient for a mixture, W is the volume of the solution, V is the volume of the hydrophobic phase and $Q^0_{water,i}$ is the initial amount of chemical i in the water.

In addition, the K_{MD}-based QSAR models were successfully utilised to quantify mixture toxicity in our previous studies (Lin et al., 2001; Lin et al., 2002), and these results indicated that the K_{MD} is one of the most effective parameters for evaluating the toxicity of mixtures.

However, it still remains unclear whether the linear relationship between hydrophobicity and toxicity exists for all PFCAs, whether the total hydrophobicity of the mixtures of PFCAs can be quantified by using K_{MD} or K_{owmix}, and whether there is a hydrophobicity-dependent QSAR that can

Table 2 – The mixture toxicity of PFCAs.

No.	Individual chemicals in mixtures	Ratio of toxic unit	$\log K_{MD}$	$\log K'_{MD}$	$\log K_{owmix}$	$\log K'_{owmix}$	TU	$\log(1/EC_{50mix})$
1	1# 2#	1:1	2.76	2.76	2.16	2.16	0.95	4.16
2	2# 4#	1:1	4.43	4.43	3.97	3.97	0.95	4.25
3	4# 9#	1:1	5.21	5.21	9.39	9.39	1.01	4.45
4	4# 10#	1:1	5.13	5.13	11.20	11.20	1.18	4.42
5	4# 11#	1:1	5.16	5.16	13.17	11.00	1.07	4.44
6	5# 10#	1:1	5.88	5.88	11.22	11.22	0.97	4.64
7	6# 9#	1:1	6.85	6.85	9.55	9.55	0.81	4.70
8	8# 11#	1:1	8.79	8.79	13.65	11.48	1.16	4.87
9	9# 10#	1:1	10.61	10.61	11.89	11.89	1.18	5.04
10	10# 11#	1:1	12.71	12.09	13.85	11.96	1.09	5.03
11	11# 12#	10:1	14.15	10.46	15.04	11.81	0.99	5.00
12	11# 12#	5:1	14.36	9.86	15.62	11.6	1.15	4.90
13	11# 12#	3:1	14.42	9.78	15.69	11.53	1.20	4.85
14	11# 12#	1:1	14.42	9.79	15.69	11.53	1.05	4.97
15	11# 12#	1:3	14.75	9.62	15.86	11.19	1.20	4.85
16	11# 12#	1:5	14.92	9.58	15.90	11.01	1.09	4.88
17	11# 12#	1:10	15.15	9.55	15.93	10.75	1.18	4.84

be used to predict PFCA mixture toxicity. These questions were addressed in the present study. Therefore, the purpose of this paper is as follows: (1) to determine both the individual and mixture toxicity of PFCAs to *Photobacterium phosphoreum*, (2) to compare K_{MD} with K_{owmix} to quantify the hydrophobicity of these mixtures and (3) to develop hydrophobicity-dependent QSARs to predict individual and/or mixture toxicity.

2. Materials and methods

2.1. Chemicals.

Twelve perfluorinated carboxylic acids (Table 1) were purchased in the highest commercially available purity from Sigma-Aldrich and Matrix; the lowest purity was 95%. Stock solutions of these compounds were prepared in 3% NaCl, diluted to a working concentration. Because of solubility problems, DMSO was used to improve the solubility of the compounds. The concentration of DMSO was less than 2% in the diluted samples.

2.2. Toxicity experiment.

The luminescent bacteria, *P. phosphoreum*, were obtained from the Institute of Soil Science, Chinese Academy of Science. Prior to toxicity testing, *P. phosphoreum* was cultured in lipid culture medium for 12 h, and then it was diluted in 3% NaCl and cultured for another 40 min at 20 °C. Next, 0.2 ml of *P. phosphoreum* suspension and 0.8 ml of the test chemicals were added to the measurement system in triplicate, providing three replicates of each concentration per individual test. Tests were performed in triplicate using 6 dilutions and 3% NaCl solution as a control. After 15 min incubation at 20 °C, the luminescence was measured with a chemiluminescent immunoassay analyser (BH9507, Beijing Hamamatsu Company). Based on the decrease in light emission from the bacteria as a result of exposure to the test chemicals, toxicity was quantified and the median effective concentration (EC_{50}) was calculated (using the probit model and reported as log ($1/EC_{50}$) in units of mol L^{-1}). The toxicity of 12 single chemicals was observed, and the results are listed in Table 1.

The tests of mixture toxicity were conducted in a similar manner as the individual chemical tests. The joint effects were presented as the sum of toxic units (TU) as follows:

$$TU = \frac{z_1}{Z_1} + \frac{z_2}{Z_2} \quad (4)$$

where z_i is the toxicant concentration and Z_i is the EC_{50} value. The combination of z_1 and z_2 results in an exact 50% response. According to the study by Broderius et al. (1995), simple addition is characterised by $TU = 1 \pm 0.2$, where $TU > 1.2$ represents antagonism and $TU < 0.8$ indicates synergism. The TU values of mixtures are presented in Table 2. Furthermore, the toxicity of mixtures was described by Eq. (5):

$$EC_{50mix} = \frac{C_{mix}}{\sum_{i=1}^{n}(C_i/EC_{50i})} \quad (5)$$

where EC_{50mix} is the effective concentration required to cause a 50% decrease in light output, C is the concentration of a substance, and the subscripts mix and *i* are the mixture and the *i*th individual chemical in the mixture, respectively. The results of the mixture toxicity tests are presented in Table 2

2.3. Statistical analyses

Statistical analyses were performed using the ORIGIN 8.0 software (OriginLab Inc.). The coefficient of determination (R^2), standard deviation (SD), F ratio, and P value were considered in testing the quality of the regression.

3. Results and discussion

3.1. The toxicity of PFCAs

3.1.1. The toxicity of single PFCAs

The toxicity of single PFCAs to *P. phosphoreum* was determined, and the results are listed in both Table 1 and Fig. 1. There is a

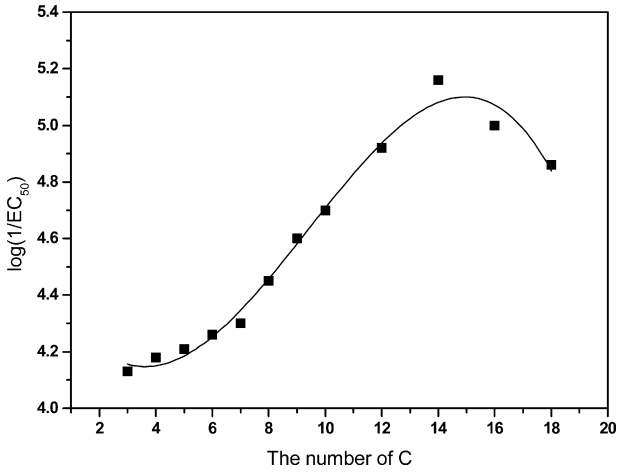

Fig. 1 – Relationship between the number of C and log ($1/EC_{50}$).

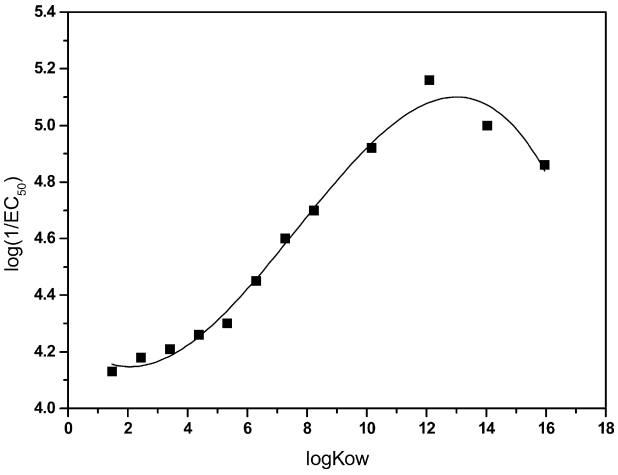

Fig. 2 – Relationship between log K_{OW} and log ($1/EC_{50}$).

tendency of increasing toxicity from C3 to C14PFCA and a tendency of decreasing toxicity from C14 to C18 PFCA. The same trend was observed in the study by Kleszczynski et al. (2007) in which the toxicity of PFCAs to HCT 116 cells was determined.

The decreasing toxicity tendency may be explained based on the hypothesis of "the maximum tolerance of the cell membrane" (Dimitrov et al., 2002). As is well known, organic chemicals are taken up from passive diffusion through the membrane to the more conservative passing of the membrane through the mechanisms of exocytosis and endocytosis. Therefore, the permeability of chemicals is controlled by "the maximum tolerance of the cell membrane", and the threshold was revealed to be 1.5 nm in the study by Dimitrov et al. (2002). Therefore, diffusion through the cell membrane is limited to the chemical molecules with a diameter that is less than the threshold of approximately 1.5 nm. In this study, the diameters of PFCAs were determined using Chemoffice 2004 (Microcal Inc.), and the results are listed in Table 1. The diameters of PFCAs (from C3 to C12) are below 1.5 nm, and the diameters of C14 PFCA, C16 PFCA and C18 PFCA are 1.5 nm, 1.7 nm and 1.9 nm, respectively. Therefore, according to the hypothesis of "the maximum tolerance of the cell membrane", one can conclude that the diffusion of C3 to C12 PFCAs through the cell membrane occurs easily, while that of C14 to C18 PFCAs is difficult. This difficult diffusion of PFCAs (from C14 to C18) leads to the decreasing toxicity tendency of C14 to C18 PFCAs presented in Fig. 1.

The increasing toxicity tendency of C3 to C14 PFCAs presented in Fig. 1 is correlated with the increasing number of carbon atoms in the perfluoroalkyl chain. Because there is a highly linear correlation between the number of carbon atoms in the perfluoroalkyl chain and their hydrophobicity, one can deduce that it is the increasing hydrophobicity that leads to the increasing toxicity tendency of C3 to C14 PFCAs. The same trend is can be observed in the data published by Kleszczynski et al. (2007).

3.1.2. *Mixture toxicity of PFCAs*

Based on the observed EC_{50} of individual PFCAs, the mixture toxicity was determined. The results are listed in Table 2. If the mixtures consisted of either the same ratio of toxic units, such as no. 1–10, or different ratios of toxic units, such as no. 11–17, there is simple addition because the range of TU values for the 17 mixtures is between 0.80 and 1.20.

Furthermore, in comparing no. 10 (consisting of #10 and #11) with no. 14 (consisting of #11, #12,), the total hydrophobicity increases from 12.71 to 14.42, but the toxicity decreases from 5.03 to 4.97. It is therefore concluded that "the maximum tolerance of the cell membrane" may exert the effect on the mixture.

3.2. *Hydrophobicity-dependent QSARs for individual PFCAs*

3.2.1. *K_{OW}-dependent QSARs for individual PFCAs*

Because the toxicity of PFCAs is highly dependent on the hydrophobicity, the relationship between log K_{OW} and log ($1/EC_{50}$) and toxicity in *P. phosphoreum* was studied (Fig. 2).

There is no linear relationship between log K_{OW} and log($1/EC_{50}$). Therefore, the polynomial equation was employed in Eq. (6) as follows:

$$\log\left(\frac{1}{EC_{50}}\right) = -5.333E^{-6}\log K_{OW}^4 - 0.001\log K_{OW}^3 + 0.032\log K_{OW}^2 - 0.115\ \log\ K_{OW} + 4.259 \quad (6)$$

$n = 12$, $R^2 = 0.980$, SD = 0.051, $F = 134.516$, $p = 0.000$.

Although the value of R^2 (0.980) in Eq. 6 indicates a high degree of correlation between log K_{OW} and log ($1/EC_{50}$), Eq. 6 is not a good prediction model because it is a complex polynomial. Therefore, this complex polynomial was simplified to the linear relationship by introducing the equivalent log K_{OW} (named as log K'_{OW}) described by Wang and Zulin (2006). The relationship between log K_{OW} and log K'_{OW} is shown in Fig. 3.

As shown in Fig. 3, assuming there are chemicals X and X' that have different partition coefficients but the same toxicity, the partition coefficient of X' can be considered to be the equivalent of X in regard to toxicity. Therefore, log K'_{OW} was referred to as the equivalent log K_{OW} by Wang and Zulin (2006). Furthermore, by using log K'_{OW} instead of log K_{OW}, he modified

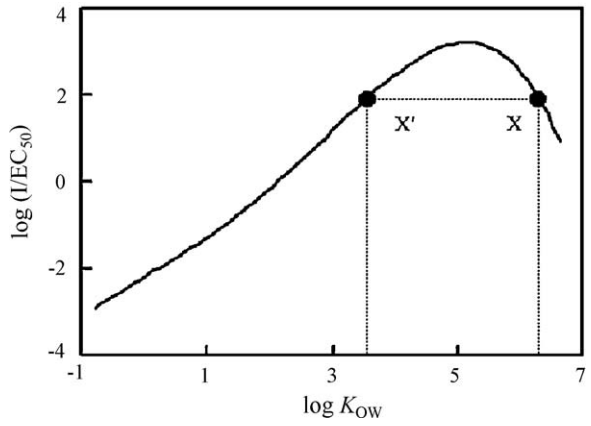

Fig. 3 – The relationship between $\log K_{OW}$ and $\log K'_{OW}$ (Wang and Zulin, 2006).

the reduced toxicity of chemical X and therefore, obtained a simple linear hydrophobicity-dependent QSAR.

In this study, the $\log K'_{OW}$ of C16 and C18 PFCA were calculated according to Fig. 1 and Eq. 6. The results are listed in Table 1, and linear hydrophobicity-dependent QSARs can be determined as follows:

$$\log\left(\frac{1}{EC_{50}}\right) = 0.099 \log K'_{OW} + 3.887 \quad (7)$$

$n = 12$, $R^2 = 0.963$, SD = 0.069, $F = 291.208$, $p = 0.000$.

Comparison of Eqs. (6) and (7) demonstrates that the introduction of $\log K'_{OW}$ greatly simplifies the complex polynomial hydrophobicity-dependent QSARs, and both K_{OW} and K'_{OW} can successfully describe the hydrophobicity of individual PFCAs.

3.2.2. K_{SD}-dependent QSARs for individual PFCAs

It has been demonstrated there is a linear relationship between $\log K_{OW}$ and $\log K_{SD}$ referred to in Eq. (2).

The equivalent $\log K_{SD}$ may predict the toxicity of individual PFCAs in a linear way as similar to $\log K'_{OW}$. Based on the K'_{OW}, the K'_{SD} was obtained by using Eq. (2) (Table 1). Based on these K'_{SD}, the relationship between $\log(1/EC_{50})$ and $\log K'_{SD}$ was studied by using Eq. (8):

$$\log\left(\frac{1}{EC_{50}}\right) = 0.099 \log K'_{SD} + 3.819 \quad (8)$$

$n = 12$, $R^2 = 0.964$, SD = 0.06818, $F = 294.535$, $p = 0.000$.

The good correlation coefficient (0.964) demonstrates that the K'_{SD} is a good parameter to predict the toxicity of individual PFCAs.

3.3. Hydrophobicity-dependent QSARs for PFCA mixtures

The hydrophobicity-dependent QSAR successfully predicted toxicity of individual PFCAs. Furthermore, the joint effects of these mixtures are only simple addition, which indicates that individual chemicals act in the same way, by the same mechanism, and differ only in their potencies. Therefore, we assumed that the toxicity of the mixtures may be related to the total hydrophobicity of the mixtures. As described previously, the total hydrophobicity of the mixtures can be quantified by using the two physicochemical parameters (K_{owmix} (Eq. (1)) and K_{MD} (Eq. (3)). The corresponding hydrophobicity-dependent QSARs for PFCA mixtures were formulated as follows.

3.3.1. K_{owmix}-dependent QSARs for mixture PFCAs

Using the K_{OW}, K_{owmix} was obtained with Eq. 1 (Table 1). Based on these K_{owmix}, the relationship between $\log(1/EC_{50})$ and $\log K_{owmix}$ was studied using Eq. (9):

$$\log\left(\frac{1}{EC_{50}}\right) = 0.055 \log K_{owmix} + 4.040 \quad (9)$$

$n = 17$, $R^2 = 0.655$ SD = 0.164, $F = 31.396$, $p = 0.000$.

The poor correlation coefficient (0.655) suggests that "the maximum tolerance of the cell membrane" may drive the mixture toxicity. Therefore, similar to K'_{OW}, K'_{owmix} was obtained using Eq. (1) (Table 1), and a K'_{owmix}-dependent QSAR for PFCA mixtures was developed using Eq. (10) as follows:

$$\log\left(\frac{1}{EC_{50mix}}\right) = 0.079 \log K'_{owmix} + 3.917 \quad (10)$$

$n = 17$, $R^2 = 0.599$, SD = 0.177, $F = 24.870$, $p = 0.000$.

However, comparison of Eq. (10) to Eq. (9) demonstrates that the introduction of K'_{owmix} does not eliminate the effect of "the maximum tolerance of the cell membrane" in the assessment of mixture toxicity. This unsuccessful improvement may be due to the three conditions assumed for calculation of K_{owmix}: well-known composition, ideal behaviour and low aqueous concentrations ($C_i^w \ll S_i^w$) (Altenburger et al., 2003). It is clear that the conditions cannot be fulfilled in all studies. In addition, there is also no experimental data to demonstrate the validity of Eq. (1). Therefore, K_{owmix} cannot be used generally.

K_{MD} may be used to describe the total hydrophobicity instead of K_{owmix} because the consistency between the calculated and the observed K_{MD} was demonstrated in our previous study (Lin et al., 2001).

3.3.2. K_{MD}-dependent QSARs for mixture PFCAs

K_{MD} was calculated using Eq. (3) (Table 2). Based on these K_{MD} values, the relationship between $\log(1/EC_{50mix})$ and $\log K_{MD}$ was studied using Eq. 11 as follows:

$$\log\left(\frac{1}{EC_{50mix}}\right) = 0.052 \log K_{MD} + 4.201 \quad (11)$$

$n = 17$, $R^2 = 0.722$, SD = 0.148, $F = 42.456$, $p = 0.000$.

The correlation coefficient ($R^2 = 0.722$) is relatively poor when compared with that in Eq. (8) ($R^2 = 0.964$). This relatively poor correlation coefficient may be partially caused by the effect of "the maximum tolerance of the cell membrane" on mixture toxicity.

To eliminate the effect of "the maximum tolerance of the cell membrane" on models of mixture toxicity, the K'_{SD} and the K'_{MD} were calculated according to Eqs. (2) and (3), respec-

Table 3 – Results of log ($1/EC_{50mix}$) for 6 other related mixtures.

Individual chemicals in mixtures	Ratio of toxic unit	$\log K_{MD}$	$\log K'_{MD}$	$\log(1/EC_{50mix})$			TU
				Obs.	Pre.	Diff	
3# 9#	1:1	4.82	4.82	4.54	4.36	0.18	0.86
3# 10#	1:1	4.51	4.51	4.40	4.33	0.07	1.20
1# 9#	1:1	4.68	4.68	4.50	4.34	0.16	0.82
9# 11#	1:1	10.52	10.52	4.92	4.93	−0.01	1.18
1# 10#	1:1	4.48	4.48	4.37	4.32	0.05	1.14
11# 12#	1:2	14.83	9.60	4.94	4.84	0.10	0.95

tively. The K_{MD}-dependent QSARs (Eq. (11)) can be rewritten as follows:

$$\log\left(\frac{1}{EC_{50}}\right) = 0.100\log K'_{MD} + 3.924 \quad (12)$$

$n = 17$, $R^2 = 0.941$, SD = 0.068, $F = 255.457$, $p = 0.000$.

The value of R^2 (0.941) in Eq. (12) indicates that the introduction of the $\log K'_{MD}$ greatly improves the quality of the K_{MD}-dependent QSARs model in Eq. (11), thus eliminating the effect of "the maximum tolerance of the cell membrane" on the mixture assessment.

In addition, the predictive capability of the model and the statistical validity of the model are confirmed by application of the six other related mixtures to the model (Table 3). The predicted log ($1/EC_{50mix}$) are plotted against the observed ones in Eq. 13. They are consistent with an $R^2 = 0.930$, SD = 0.075 and $F = 67.372$ at a level of significance $p = 0.001$.

$$\log\left(\frac{1}{EC_{50mix}}\right)\text{pre.} = 1.086\log\left(\frac{1}{EC_{50mix}}\right)\text{obs.} - 0.489 \quad (13)$$

$n = 6$, $R^2 = 0.930$, SD = 0.075, $F = 67.372$, $p = 0.001$.

Because the six mixtures are randomly composed of the 12 individual chemicals and the agreement between the observed log ($1/EC_{50mix}$) and that predicted by the model is generally satisfactory, the model therefore can be used to predict the mixture toxicity of PFCAs, and K_{MD} can be used to describe the total hydrophobicity instead of K_{owmix}.

4. Conclusion

1. There was a tendency of increasing toxicity from C3 to C14 PFCAs and a tendency of decreasing toxicity from C14 to C18 PFCA. Based on the K'_{OW} and K'_{SD}, there is a good linear relationship between the toxicity and the hydrophobicity of the PFCAs.
2. The mixture effect of the PFCAs is simple addition because the range of TU values for the mixture is between 0.80 and 1.20. Equivalent $\log K_{MD}$ can be used to predict the mixture toxicity in a linear model, but equivalent $\log K'_{OW}$ cannot, which shows that K_{MD} is the more suitable parameter to describe the hydrophobicity of the mixture.
3. The QSAR model for mixture toxicity is as follows: $\log(1/EC_{50mix}) = 0.052\log K_{MD} + 4.201$. This model is potentially useful for environmental pollutant control and ecological risk assessment.

Conflict of interest

Nothing declared.

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