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Modelling bioconcentration of pesticides in fish using biopartitioning micellar chromatography

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Abstract

Ecotoxicity assessment is essential before placing new chemical substances on the market. An investigation of the use of the chromatographic retention ($\log k$) in biopartitioning micellar chromatography (BMC) as an in vitro approach to evaluate the bioconcentration factor (BCF) of pesticides in fish is proposed. A heterogeneous set of 85 pesticides from six chemical families was used. For pesticides exhibiting bioconcentration in fish (experimental $\log \mathrm{BCF} > 2$), a quantitative retention–activity relationships (QRAR) model is able to perform precise $\log \mathrm{BCF}$ estimations of new pesticides. Considering the present data, the results based on $\log k$ seem to be more reliable than those from available software (BCFWIN and KOWWIN) and from $\log P$ (quantitative structure–activity relationships (QSAR)). It is also possible to perform risk assessment tasks fixing a threshold value for $\log k$, which substitute two common threshold values, $\log P$ and experimental $\log \mathrm{BCF}$, avoiding the experimental problems related with these two parameters.

Keywords: Bioconcentration factor; Pesticides fish bioaccumulation; Biopartitioning micellar

1. Introduction

Pesticides are used in agricultural treatments to reduce the negative impact of plagues. Despite this benefit, the use of this kind of chemicals must be controlled because an important fraction of these pesticides are released into the environment presenting a potential hazard risk. The aquatic organisms can accumulate chemicals present in the aquatic media. Some chemicals may be found only at low levels in various tissues, whereas others may build up to significant concentrations [1]. The tendency of the organism to bioaccumulate is measured by the bioconcentration factor (BCF) which is formally defined as the equilibrium ratio of the concentration of the substance in the exposed organism to the concentration of the dissolved substance bioavailable in the surrounding aquatic environment [2,3].

BCF is important to indicate the future response of organisms to a toxic substance, but also to evaluate the biomagnification process (i.e. the accumulation of chemical in the trophic chain due to dietary absorption) [1,3]. BCF can be estimated by means of in vivo test. Fishes with an average lipid content of 4.8% are good model animals for bioconcentration studies [4]. The European Union (EU) proposes an experimental method for BCF determination in fishes [5]. This assay is divided into two steps, the uptake phase where the fishes are exposed to the test substance and the clearance phase where the fishes are transferred to a medium free of the test substance. Experimental BCF values are compulsory to satisfy legislative protocols requirements, as for instance Directive 93/21/EEC [6].

Unfortunately, the experimental determination of BCF is time consuming, difficult, expensive and measuring the BCF of the many thousands of chemical substances that are of potential interest, simply is not possible [2,7]. Since experimental data are not available for all chemicals in use, many

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researchers tend to use estimation methods to supply the missing data. Numerous correlations have been developed relating BCF values to the *n*-octanol–water partition coefficient (log *P*) [8]. Unfortunately, not all the BCF values have always a good correlation with log *P* with any kind of compound, and the application of these methods is limited by the availability of parameter data [9]. On the other hand, log *P* data are employed in legislative protocols as a 'long-term adverse effects in the aquatic environment' criterion [6].

Chromatographic analytical determination of organic compounds has served as the final step to quantify bioconcentration factors in different organisms after in vivo test, however, to our knowledge there are only two applications involving direct in vitro evaluation of the BCF by means of the chromatographic retention of the compounds [10,11]. In the first case, the prediction of bioconcentration potential of organic compounds using partition coefficients derived from reversed phase thin layer chromatography is performed. In the second application, biopartitioning micellar chromatography (BMC), a mode of reversed phase micellar liquid chromatography, is used to relate the retention factor with the log BCF estimations obtained from a computer algorithm (BCFWIN software).

The aim of this paper is to study the use of the pesticides retention in BMC (log *k*), as independent variable, as an alternative in vitro approach to modelling their bioconcentration in fish, using in vivo experimental data (log BCF, as dependent variable), thus permitting their risk assessment consistent with legislative requirements. For this purpose, a heterogeneous data set of 85 pesticides formed by six families of pesticides was used. Quantitative retention–activity relationships (QRAR) for the BCF estimations is obtained. The results obtained using log *k* are compared with those using the BCFWIN software and an empirical quantitative structure–activity relationship (QSAR) model based on log *P*.

2. Experimental

2.1. Instrumental and measurements

A Hewlett-Packard HP 1100 chromatograph with an isocratic pump, an UV–vis detector, a column thermostat and an autosampler with a 20 μL loop were employed to obtain the retention values. Data acquisition and processing were performed by means of a HP Vectra XM computer (Amsterdam, The Netherlands) equipped with HP-Chemstation software (A.07.01 [682] ©HP 1999). Two Kromasil C_{18} columns (5 μm, 150 mm × 4.6 mm i.d.; Scharlab, Barcelona, Spain) and (5 μm, 50 mm × 4.6 mm i.d.; Scharlab), for less and more hydrophobic compounds, respectively, were used. The mobile phase flow rates were 1.0 and 1.5 ml min^{-1}, for the 150 and 50 mm length columns, respectively. The detection was performed in UV at 245 nm for carbamates and phenylureas, at 230 nm for phenoxyacids and triazines, at 224 nm for organochlorines and at 220 nm for organophosphorous pesticides. All the assays were carried out at 25 °C.

2.2. Reagents and standards

Micellar mobile phases were prepared by dissolving the adequate amount of polyoxyethylene(23)lauryl ether (Brij35, Acros Chimica, Geel, Belgium) in aqueous solutions of 0.05 M phosphate buffer to get a final surfactant concentration of 0.02, 0.04 and 0.06 M. The buffer solution was prepared with sodium dihydrogen phosphate (reagent grade, Scharlab). The pH was potentiometrically adjusted at 7.0 by addition of sodium hydroxide (97%, purissimum, Panreac, Barcelona, Spain) aqueous solutions.

Pesticides were obtained from different sources: aldoxycarb, molinate, pebulate and methoprotryn from Riedel de Haën (Seelze, Germany), 4-CPA from Sigma (St. Louis, MO, USA), 2-PPA, 2,4-DCPPA and 2,4,5-TCPPA from Chem Service (West Chester, PA, USA), MCPP, 3-CPPA and 4-CPPA from Aldrich (Milwauke, Wl, USA), DC, MCPA, 2,4-D, MCPB, 2,4,5-T, trichlorfon, dimethoate, methidathion, malathion, mecarbam, pirimiphos-methyl, chlorpyrifos-methyl, diazinon, fenthion, chlorpyrifos, pirimicarb, benomyl, carbaryl, benfuracarb, dicloran, dicofol and all phenylureas except linuron and thiazafluron, from Dr. Ehrenstorfer (Augsburg, Germany). The other pesticides were obtained from The Superior Polytechnic Centre of Engineers (University of Zaragoza, Zaragoza, Spain).

Working solutions were prepared by dissolving 0.1–0.5 mg of pesticide in 100 μL of 0.04 M Brij35 or acetonitrile (reagent grade, Scharlab), except in the case of commercial solutions of pesticides, where 100 μL were taken. In all cases, a 0.04 M Brij35 solution was added to a final volume of 2 mL. Barnstead E-pure deionized water (Sybron, Boston, MA, USA) was used throughout. The mobile phases and the solutions injected into the chromatograph were vacuum filtered through 0.45 μm Nylon membranes (Micron Separations, Westboro, MA, USA).

2.3. Software and data processing

Microsoft® Excel 2000 software (Microsoft Corporation) was used for data processing. All other calculations were performed using routines developed in MATLAB 5.3 (Matlab Ver. 5.3.0.10183 (R11), ©The Mathwoks Inc., Natick, MA).

BCFWIN (version 2.14) and KOWWIN software (version 1.66) were used for BCF and log *P* estimations, respectively. These programmes are integrated in the EPI Suite software (developed by Syracuse Research Corporation for the US Environmental Protection Agency (EPA)) [12].

2.4. Retention factor estimations

The retention factor (*k*) of pesticides was estimated according to an approach described elsewhere [13]:

$$k = \left[\left(\frac{t_R}{t_{R(REF)}}\right)(1 + k_{REF})\right] - 1 \quad (1)$$

where t_R is the experimental retention time of the pesticide assayed and $t_{R(REF)}$ is the experimental retention time of a reference compound (acetanilide) injected during the working session. k_{REF} is the retention factor of acetanilide, previously established for the experimental conditions assayed (surfactant concentration and temperature) and was considered constant. The use of this approach provides retention factor estimations more reliable and easier to obtain than the classical estimations based on the measurement of the dead time (actually the gross hold-up time [14]). For instance, this approach reduces the impact of changing the column and mobile phase flow rate, among other experimental factors, on the k estimations [13].

2.5. Data

The 85 pesticides data set is shown in Table 1 . The table includes the experimental retention data ($\log k_2$, $\log k_4$ and $\log k_6$), experimental and estimated hydrophobicity information ($\log P$ and E–$\log P$) and estimated bioconcentration information (E–log BCF). In addition, available experimental BCF data, from ECOTOX [15] and HSDB [16] databases, have been included (log BCF). For most pesticides, various experimental BCF values were available, incorporating different kind of fishes and protocols. In these cases, the median of the log BCF values was used as a robust index of the BCF in fish. However, it should not be forgotten that such experimental log BCF values represent a heterogeneous data set.

3. Results and discussion

The scoring of the bioconcentration rate or bioaccumulation potential of chemicals is generally based on BCF data for fish, or if such data are not available, generally on $\log P$ data, according to different threshold values [17]. According to the Directive 93/21/EEC [6], related to the classification, packaging and labelling of dangerous substances, compounds with $\log P \geq 3$ and log BCF > 2 may cause long-term adverse effects in the aquatic environment and they are labelled as R53 for their risk identification. In Table 1, due to the lack of experimental data, particularly log BCF (with 50 non-available data), only 61 out of the 85 pesticides could be assessed as R53-pesticides ($\log P \geq 3$ and log BCF > 2) or non-R53 pesticides ($\log P < 3$ or $\log P \geq 3$ but log BCF ≤ 2). This is a general problem in the literature [17] and, in order to overcome it, several approaches can be used. Some of them are discussed in the following sections.

3.1. Software-based BCF estimations

Fast BCF and $\log P$ estimations can be obtained using available software (i.e. BCFWIN and KOWWIN). Table 1 provides these data (E–log BCF and E–$\log P$, respectively) and Fig. 1a shows the E–log BCF versus E–$\log P$ plot for all pesticides, labelled according to variable FAM. The thresh-

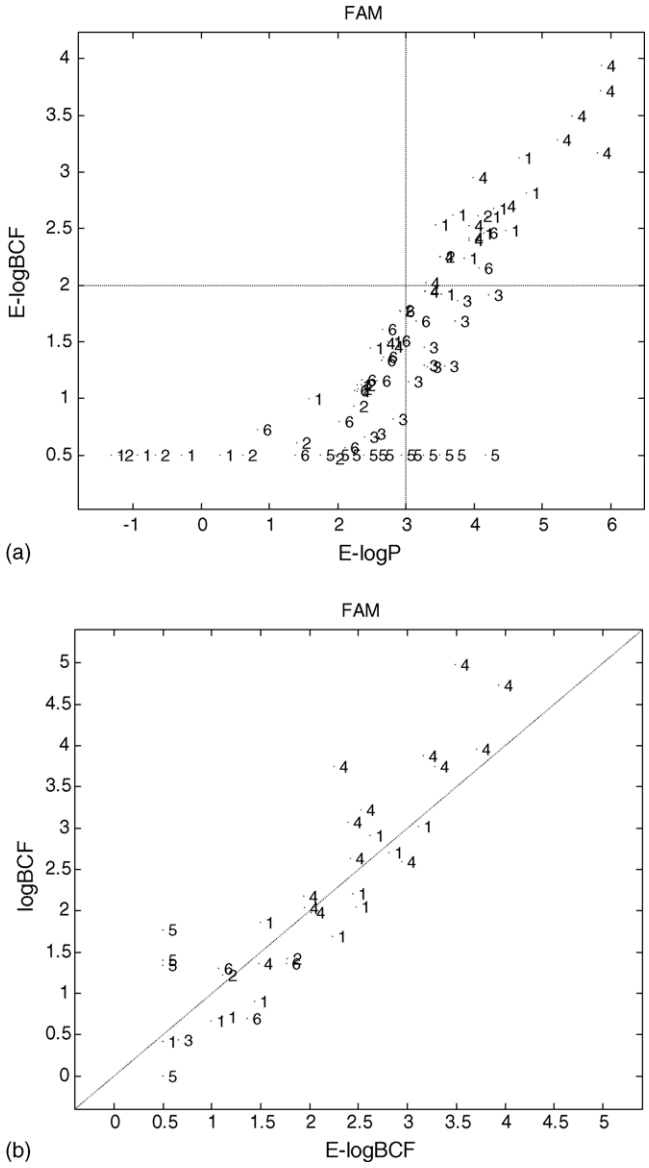

Fig. 1. (a) E–log BCF vs. E–$\log P$ plot showing the log BCF – $\log P$ relationships determined by the rules imposed by BCFWIN and KOWWIN software. The threshold values (log BCF = 2 and $\log P = 3$) corresponding to the Directive 93/21/EEC are included. (b) Experimental log BCF vs. estimated E–log BCF plot. The ideal line log BCF = E–log BCF is included. The plots show the pesticides labelled according to the chemical family (FAM).

old values ($\log P = 3$ and log BCF = 2) corresponding to the EC Directive 93/21/EEC are also included. The log BCF-$\log P$ relationships are determined by the rules imposed by BCFWIN and KOWWIN software. As can be observed, some low hydrophobic FAM = 1- and FAM = 2-pesticides and all the FAM = 5-pesticides (ionic compounds) are set to E–log BCF = 0.5 automatically by these algorithms. This is not a critic aspect since such compounds are assumed not to bioconcentrate. For the rest of pesticides, the software provides an apparent linear relationship between E–log BCF and E–$\log P$, almost independent of the FAM of the compound.

Table 1
Retention, hydrophobicity and BCF data of pesticides

CAS	Compound	*N*	FAM	log k_2	log k_4	log k_6	log *P*	*E*–log *P*	log BCF	*E*–log BCF
2157-98-4	Monocrotophos	1	1	0.321	0.207	0.255	−0.20	−1.31	–	0.500
10265-92-6	Methamidophos	2	1	0.117	0.007	0.095	−0.80	−0.93	–	0.500
52-68-6	Trichlorfon	3	1	0.412	0.722	0.296	0.51	−0.28	–	0.500
60-51-5	Dimethoate	4	1	1.068	0.941	0.860	0.78	0.28	0.410	0.500
950-37-8	Methidathion	5	1	1.993	1.748	1.546	2.20	1.58	0.663	0.994
121-75-5	Malathion	6	1	2.153	1.893	1.686	2.37	2.29	0.716	1.117
2595-54-2	Mecarbam	7	1	2.131	1.885	1.693	–	2.29	–	1.063
732-11-6	Phosmet	8	1	1.979	1.714	1.503	2.78	2.48	0.903	1.441
298-00-0	Parathion-methyl	9	1	2.083	1.817	1.604	2.86	2.75	1.851	1.502
29232-93-7	Pirimiphos-methyl	10	1	2.384	2.116	1.894	4.20	3.44	–	2.534
2642-71-9	Azinphos-ethyl	11	1	2.156	1.887	1.672	3.40	3.52	–	1.918
5598-13-0	Chlorpyrifos-methyl	12	1	2.353	2.085	1.863	4.31	3.69	2.904	2.619
333-41-5	Diazinon	13	1	2.379	2.112	1.896	3.81	3.86	1.681	2.234
55-38-9	Fenthion	14	1	2.234	1.965	1.745	4.09	4.09	2.204	2.449
13067-93-1	Cyanofenphos	15	1	2.307	2.037	1.813	4.29	4.20	–	2.603
2310-17-0	Phosalone	16	1	2.448	2.179	1.949	4.38	4.29	–	2.673
56-72-4	Coumaphos	17	1	2.282	2.014	1.790	4.13	4.47	2.041	2.480
2921-88-2	Chlorpyrifos	18	1	2.569	2.300	2.073	4.96	4.66	3.019	3.119
57018-04-9	Tolclofos-methyl	19	1	2.305	2.037	1.818	4.56	4.77	2.696	2.811
23135-22-0	Oxamyl	20	2	0.326	0.169	0.258	−0.48	−1.20	–	0.500
1646-88-4	Aldoxycarb	21	2	0.296	0.191	0.239	−0.57	−0.67	–	0.500
16752-77-5	Methomyl	22	2	0.511	0.409	0.414	0.60	0.61	–	0.500
23103-98-2	Pirimicarb	23	2	1.553	1.354	1.248	1.70	1.40	–	0.609
114-26-1	Propoxur	24	2	1.446	1.280	1.163	1.52	1.90	–	0.470
17804-35-2	Benomyl	25	2	1.345	1.149	1.009	2.12	2.24	–	0.932
1563-66-2	Carbofuran	26	2	1.511	1.328	1.196	2.32	2.30	–	1.086
63-25-2	Carbaryl	27	2	1.735	1.493	1.297	2.36	2.35	1.216	1.117
2212-67-1	Molinate	28	2	2.050	1.814	1.644	3.21	2.91	1.415	1.772
1114-71-2	Pebulate	29	2	2.399	2.130	1.925	3.83	3.51	–	2.249
82560-54-1	Benfuracarb	30	2	2.516	2.257	2.024	4.30	4.06	–	2.611
122-34-9	Simazine	31	3	1.609	1.394	1.239	2.18	2.40	0.433	0.659
21725-46-2	Cyanazine	32	3	1.647	1.415	1.244	2.22	2.51	–	0.689
1014-69-3	Desmetryn	33	3	1.721	1.566	1.340	2.38	2.82	–	0.813
841-06-5	Methoprotryn	34	3	1.849	1.674	1.445	2.82	3.04	–	1.151
4658-28-0	Aziprotryne	35	3	2.043	1.790	1.594	3.00	3.27	–	1.290
5915-41-3	Terbuthylazine	36	3	1.995	1.736	1.536	3.22	3.27	–	1.452
834-12-8	Ametryne	37	3	1.889	1.698	1.470	2.98	3.32	–	1.275
1610-18-0	Prometon	38	3	1.837	1.599	1.432	2.99	3.57	–	1.282
7287-19-6	Prometryn	39	3	2.077	1.815	1.613	3.51	3.73	–	1.683
886-50-0	Terbutryne	40	3	2.084	1.845	1.622	3.74	3.77	–	1.860
4147-51-7	Dipropetryn	41	3	2.207	1.942	1.731	3.81	4.22	–	1.914
108-90-7	Chlorobenzene	42	4	2.060	1.820	1.632	2.84	2.64	1.362	1.487
99-30-9	Dicloran	43	4	1.931	1.668	1.457	2.80	2.76	–	1.456
95-50-1	1,2-Dichlorobenzene	44	4	2.126	1.867	1.662	3.43	3.28	2.176	1.941
106-46-7	1,4-Dichlorobenzene	45	4	2.240	1.982	1.780	3.44	3.29	2.034	1.949
541-73-1	1,3-Dichlorobenzene	46	4	2.195	1.952	1.746	3.53	3.30	1.971	2.018
33213-65-9	β-Endosulfan	47	4	2.424	2.176	1.923	3.83	3.50	3.744	2.249
120-82-1	1,2,4-Trichlorobenzene	48	4	2.311	2.046	1.835	4.02	3.93	3.063	2.395
108-70-3	1,3,5-Trichlorobenzene	49	4	2.392	2.128	1.919	4.19	3.93	3.217	2.526
87-61-6	1,2,3-Trichlorobenzene	50	4	2.206	1.944	1.730	4.05	3.93	2.633	2.419
510-15-6	Chlorbenzylate	51	4	2.401	2.130	1.903	4.74	3.99	2.588	2.950
5836-10-2	Chlorpropylate	52	4	2.509	2.239	2.009	–	4.41	–	2.696
608-93-5	Pentachlorobenzene	53	4	2.504	2.236	2.021	5.17	5.22	3.740	3.281
118-74-1	Hexachlorobenzene	54	4	2.623	2.354	2.137	5.73	5.86	3.949	3.712
3547-04-4	DDE	55	4	2.769	2.497	2.262	–	5.44	4.975	3.488
72-54-8	DDD	56	4	2.693	2.420	2.184	6.02	5.87	4.720	3.935
115-32-2	Dicofol	57	4	2.429	2.413	2.175	5.02	5.81	3.875	3.166
940-31-8	2-PPA	58	5	0.175	−0.026	0.090	–	1.75	–	0.500
122-88-3	4-CPA	59	5	1.218	0.863	0.836	2.25	1.97	–	0.500
1918-00-9	DC	60	5	0.346	0.145	0.246	2.21	2.14	–	0.500

Table 1 (*Continued*)

CAS	Compound	*N*	FAM	log k_2	log k_4	log k_6	log *P*	*E*–log *P*	log BCF	*E*–log BCF
101-10-0	3-CPPA	61	5	0.792	0.527	0.562	–	2.39	–	0.500
3307-39-9	4-CPPA	62	5	0.812	0.544	0.573	2.31	2.39	–	0.500
94-74-6	MCPA	63	5	1.207	0.844	0.816	3.25	2.52	0.000	0.500
94-75-7	2,4-D	64	5	1.211	0.855	0.833	2.81	2.62	1.338	0.500
93-65-2	MCPP	65	5	1.291	0.904	0.876	3.13	2.94	–	0.500
120-36-5	2,4-DCPPA	66	5	1.317	0.936	0.908	3.43	3.03	–	0.500
93-76-5	2,4,5-T	67	5	1.482	1.067	1.002	3.31	3.26	1.398	0.500
94-81-5	MCPB	68	5	1.702	1.251	1.135	–	3.50	–	0.500
93-72-1	2,4,5-TCPPA	69	5	1.556	1.135	1.062	3.80	3.68	1.763	0.500
113158-40-0	Fenoxaprop-P	70	5	1.552	1.084	1.027	–	4.17	–	0.500
101-42-8	Fenuron	71	6	1.012	0.886	0.823	0.98	1.38	–	0.500
150-68-5	Monuron	72	6	1.591	1.372	1.194	1.94	2.03	–	0.794
19937-59-8	Metoxuron	73	6	1.435	1.232	1.073	1.64	2.11	–	0.563
1746-81-2	Monolinuron	74	6	1.769	1.535	1.340	2.30	2.26	1.301	1.071
2164-17-2	Fluometuron	75	6	1.789	1.554	1.352	2.42	2.36	–	1.163
15545-48-9	Chlorotoluron	76	6	1.736	1.486	1.294	2.41	2.58	–	1.156
18691-97-9	Methabenzthiazuron	77	6	1.748	1.490	1.292	2.64	2.65	–	1.333
330-54-1	Diuron	78	6	1.800	1.543	1.334	2.68	2.67	0.690	1.364
34123-59-6	Isoproturon	79	6	1.770	1.531	1.349	2.84	2.87	–	1.510
330-55-2	Linuron	80	6	1.920	1.653	1.443	3.20	2.92	1.362	1.764
13360-45-7	Chlorbromuron	81	6	1.936	1.672	1.456	3.09	3.15	–	1.679
1982-47-4	Chloroxuron	82	6	1.955	1.689	1.473	3.70	4.08	–	2.149
555-37-3	Neburon	83	6	2.218	1.957	1.725	4.10	4.15	–	2.457
3766-60-7	Buturon	84	6	2.033	1.776	1.558	3.00	2.66	–	1.610
25366-23-8	Thiazafluron	85	6	1.735	1.532	1.343	1.85	0.83	–	0.725

N (identification number) FAM (chemical family of pesticides): (1) organophosphorous; (2) carbamates; (3) triazines; (4) organochlorines; (5) phenoxyacids; (6) phenylureas log k_2, log k_4 and log k_6 (logarithm of the retention factors obtained with 0.02, 0.04 and 0.06 M Brij35 mobile phases, respectively) log *P* and *E*–log *P* (experimental and estimated by KOWWIN values of the octanol–water partition coefficient, respectively) log BCF and *E*–log BCF (experimental from ECOTOX [15] and HSDB [16] and estimated BCFWIN values of the logarithm of the bioconcentration factors, respectively). Pesticide abbreviations: DDE, dichlorodiphenyldichloroethylene; DDD, dichlorodiphenyldichloroethane; 2-PPA, 2-phenoxypropionic acid; 4-CPA, 4-chlorophenoxyacetic acid; DC, 2-methoxy-3,6-dichlorobenzoic acid; 3-CPPA, 2-(3-chlorophenoxy) propionic acid; 4-CPPA, 2-(*p*-chlorophenoxy) propionic acid; MCPA, 2-methyl-4-chlorophenoxyacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; MCPP, 2-(2-methyl-4-chlorophenoxy) propionic acid; 2,4-DCPPA, 2,4-dichlorophenoxypropionic acid; 2,4,5-T, (2,4,5-trichlorophenoxy) acetic acid; MCPB, 4-(2-methyl-4-chlorophenoxy) butyric acid; 2,4,5-TCPPA, 2-(2,4,5-trichlorophenoxy) propionic acid.

Fig. 1b shows the log BCF versus *E*–log BCF plot for the available experimental log BCF values in Table 1, including the ideal line log BCF = *E*–log BCF. As before, pesticides are labelled according to variable FAM. With the exception of FAM = 5-pesticides, the estimated *E*–log BCF values agree moderately well to the experimental log BCF ones. However, the larger differences correspond to the pesticides with large BCF values, for which the estimated values are lower than the experimental ones. This suggests a limited usefulness of BCFWIN precisely for the most risky pesticides used in this study.

3.2. BCF estimations based on QSAR

Many models for predicting bioconcentration factors for organic chemical are based on linear and bilinear relationships between log BCF and log *P* [2,18,19]. However, false negative estimations or inadequacy for legislative purposes, associated to the use of QSAR models, have been reported [17]. On the other hand, it is difficult to find experimental log *P* values for all the compounds of interest and, since these values are difficult to obtain and they become unreliable if log $P > 4$ [20], log *P* estimations based on computer algorithms are used alternatively. However, assignment of probable error to the calculation of log *P* is very difficult [21]. Table 1 allows the comparison between the variables log *P* (experimental) and *E*–log *P* (estimated by KOWWIN software). It can be observed *E*–log *P* − log *P* differences from −1 (compounds $N = 1, 85$) to +0.8 (compound $N = 57$). This evidences the uncertainty associated with the use of log *P* data.

Fig. 2 shows the log BCF versus log *P* plot. The results resemble to some extent those of Fig. 1a. For ionic pesticides (FAM = 5) the log BCF estimations based on log *P* are higher than those based on the BCFWIN criteria. The reason is that the log *P* used corresponds to the neutral molecule, ignoring the degree of ionization of these pesticides. This fact could introduce doubts with respect to the possible bioaccumulation of some of these compounds. However, their corresponding log BCF values are under the log BCF = 2 threshold value, conferring less importance, in terms of risk, to them. For log $P > 2$, it is possible to describe a linear log BCF − log *P* relationship, which could serve to propose a QSAR model. However, some data dispersion is observed and even an atypical observation (β-endosulfan; a FAM = 4-pesticide with $N = 47$ in Table 1) is evident.

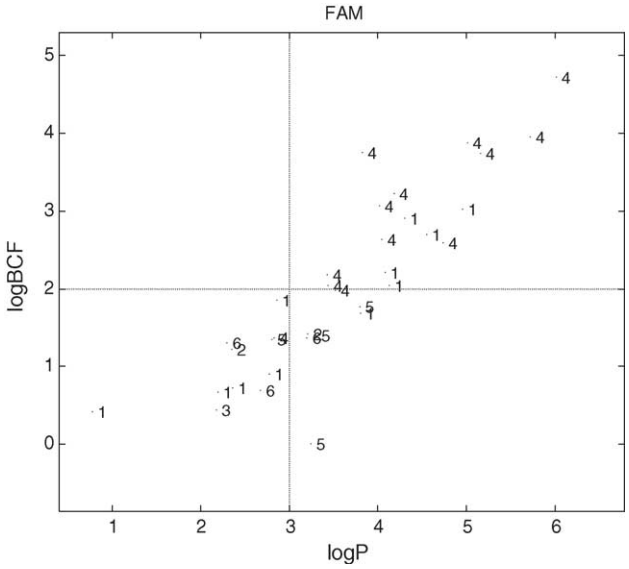

Fig. 2. log BCF – log P relationship from experimental values. The threshold values (log BCF = 2 and log P = 3) corresponding to the Directive 93/21/EEC are included. Pesticides are labelled according to the chemical family (FAM).

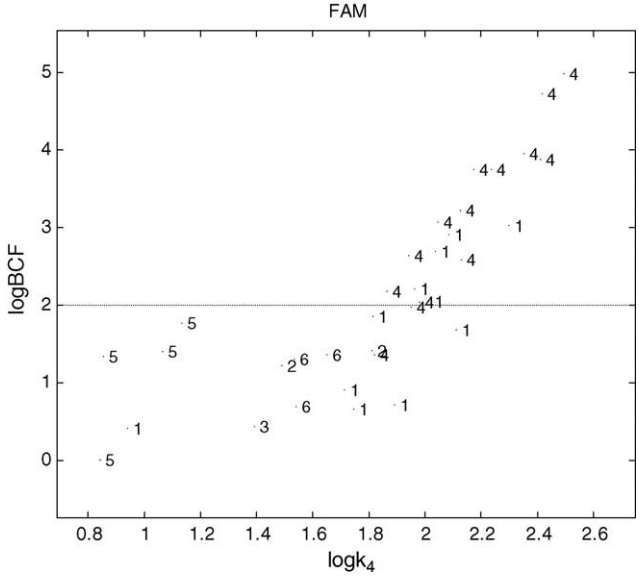

Fig. 3. log BCF – log k_4 relationship from experimental values. The threshold value (log BCF = 2) corresponding to the Directive 93/21/EEC is included. Pesticides are labelled according to the chemical family (FAM).

3.3. BCF estimations based on QRAR

Alternatively to log P, log k values can be used as independent parameter to perform QRAR models or QSAR models using log k with other descriptors. Some advantages of the use of log k instead of log P have been reported [11]. For instance, log k data are experimental values, much easier to obtain than experimental log P data and they exhibit high precision, introducing controlled uncertainty to the model [12]. Selecting the adequate chromatographic modality deserves special attention. Micelles have proven to be adequate chemical models for biomembranes mainly due to their amphiphilic and anisotropic properties [22–24]. The use of retention data obtained in a chromatographic system constituted by polvoxvethvlene(23)laurvl ether (Brij35) in concentration above the critical micellar concentration solutions as micellar mobile phases and a C_{18} reversed stationary phase, under the adequate experimental conditions, has proven to be very useful for describing the biopartitioning of chemicals in biomembranes [25–27].

This approach can be used alternatively to traditional log P measurements due to its speed, improved reproducibility and low consumption of test chemicals. In addition, some typical requirements for experimental log P determinations [22,28,29], as for instance high purity chemicals and additional analytical quantifications, are not necessary in BMC, since it conserves the intrinsic advantages of liquid chromatography.

Fig. 3 shows the log BCF versus log k_4 plot. As in the case of Fig. 2, the results resemble those in Fig. 1a. However, the location of ionic pesticides (FAM = 5) is different respect to that in Fig. 2. This suggests that log k_4 accounts not only for hydrophobicity but also for the degree of ionization of these pesticides; an issue already described in the literature [24,30].

In contrast to Fig. 2, Fig. 3 reveals larger dispersion at low BCF values (the less critic zone), but more adequate linear relationship for high (the most critic zone) BCF values using log k_4 than using log P. These results could be explained in terms of lower log k_4 contribution to the uncertainty of that relationship. Fig. 3 suggests that for log BCF > 2 (dangerous pesticides in long-term sense), the log BCF – log k_4 relationship can be expressed in terms of a QRAR model:

$$\log\ \mathrm{BCF} = -6.1(\pm 2.4) + 4.3(\pm 1.1)\log\ k_4 \qquad (2)$$

where $n = 17$, $r^2 = 0.82$, $F = 67$, $p < 0.0001$, RMSEC = 0.37, RMSECV = 0.40.

Here, intervals are the confidence limits at 95% confidence level. From a practical point of view, Eq. (2) is applicable to pesticides with log k_4 values larger than 1.85. This affects to part of the neutral compounds (the ionic pesticides are always less retained ‘than the neutral ones in BMC’, as indicated in Fig. 3, and are usually considered low-BCF compounds, as Fig. 1 indicates). For compounds with log k_4 < 1.85 log BCF values <2 are expected, but not linearly related to log k_4, which agree with the results in Fig. 1.

This model can be compared with that obtained using log P as independent variable for the same compounds (excluding the compound $N = 55$ for which no experimental log P value is available):

$$\log\ \mathrm{BCF} = -0.6(\pm 1.8) + 0.8(\pm 0.4)\log\ P \qquad (3)$$

where $n = 16$, $r^2 = 0.59$, $F = 20$, $p = 0.0005$, RMSEC = 0.49, RMSECV = 0.56.

This indicates that log k_4 fits better the actual log BCF data set (better regression statistics as coefficient of determination,

r^2, modelled-to-residual variance ratio, F and root-mean-square error in calibration, RMSEC) but also that the log k_4-model has higher predictive ability (lower root-mean-square error in cross-validation, RMSECV) than log P-model. These results could be partially explained in terms of higher log P contribution to the uncertainty of the QSAR model (Section 3.2).

As an example of the application of the QRAR model (Eq. (2) approach), the bioconcentration factor for benzofuracarb ($N = 30$), for which experimental log BCF value is not available was estimated. The estimated log BCF value by means of Eq. (2) is 3.6 ± 0.2 (confidence limit = 95%). On the other hand, the estimated (BCFWIN) value is E–log BCF = 2.6 (uncertainty not available), one unit lower than the previous one, and the estimation using Eq. (3) is 2.9 ± 0.3 (confidence limit = 95%). This example demonstrated that the approaches are not equivalent, thus affecting the decision-making step in the risk assessment studies. For instance, it is widely accepted that chemicals are classified as 'bioaccumulative' when log BFC is in the range 3.3–3.7 and 'very bioaccumulative' when log BFC > 3.7 [31]. Accordingly, benzofuracarb would be classified as 'bioaccumulative', close to the 'very bioaccumulative' threshold, only in the case of approach based on log k_4.

3.4. R53 assessment based on QRAR

It is possible to convert the EU Directive 93/21/EEC threshold values related to log P and the experimental log BCF values in a single threshold value based on log k_4 for long term adverse effects assessment (i.e. R53 classification). Pesticides with log BCF > 2 were related to log k_4 in Section 3.3 and according to Eq. (2), log BCF = 2 corresponds to log $k_4 = 1.884$. Excluding FAM = 5-pesticides, for which log P is not a consistent index of bioconcentration (see Section 3.2), log $P > 3$ pesticides can be approximately related to log $k_4 = 1.75$. A single log k_4 threshold value for R53 assessment could be based on an intermediate value. Taking into account the uncertainty associated with the log P values, we suggests the use of log $k_4 = 1.85$ as unique threshold value for pesticides risk assessment.

Fig. 4 shows the classification in non-R53- or R53-pesticedes based on the log $k = 1.85$ threshold for all pesticides in Table 1. Attending to the 61 pesticides that can be assessed from experimental log BCF and log P data (symbols '◯' for non-R53 and '□' for R53), three non-R53-pesticides would be misclassified, but all R53-pesticides would be correctly classified. Therefore, the percentage of pesticides correctly classified are 95.1%, using the log $k = 1.85$ threshold value. These figures are satisfactory considering the heterogeneity of the experimental log BCF data and the intrinsic uncertainty of log P data. Therefore, the R53 assessment of the 24 pesticides that cannot be assessed due to the lack of experimental log BCF and/or log P (symbol '△' in Fig. 4), can be estimated by means log k_4 data, in a reliable way.

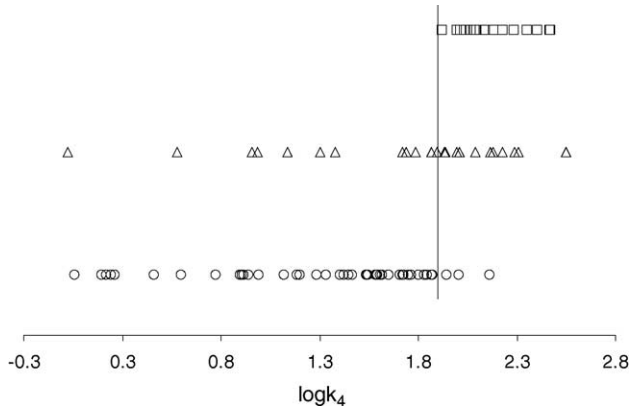

Fig. 4. Distribution along log k_4 of the pesticides labelled according to the Directive 93/21/EEC recommendations: (◯) non-R53; (△) unknown (□) R53, based on the experimental log P and log BCF values. Solid line refers to log $k_4 = 1.85$.

Similar results were obtained working with retention data obtained at other surfactant concentrations (log k_2 and log k_6), which validates the experimental log k values used. Obviously, changes in the log k-threshold value are observed, which becomes larger for log k_2 and lower for log k_6 data, respect to that from log k_4, due to the decrease of pesticides retention with the increase of Brij35 concentration.

4. Conclusions

The bioconcentration of 85 pesticides in fish is studied by means of BMC. For the first time, the retention factor of compounds in an HPLC modality was related to experimental BCF data. The results for six families of pesticides suggest that log k, a single descriptor, is efficient in representing the bioconcentration process in fish. Particularly, for the most retained pesticides (i.e. log $k_4 > 1.85$), it is possible to use a QRAR model to perform precise log BCF estimations of new pesticides, and probably other ionic and non-ionic organic compounds.

From a quantitative point of view, this model seems to be a practical alternative in vitro method to estimate the bioconcentration factor, avoiding the problems associated with the use of log P data. Considering the present data, QRAR estimations are more reliable than those obtained using the available software BCFWIN and KOWWIN and those based on a QSAR model using log P. From a qualitative point of view, the measures of log k_4 seem to be adequate in order to be incorporated in a hazard assessment strategy, for instance, they have been proven to be adequate to evaluate the R53 category of pesticides based on the threshold value log $k_4 = 1.85$.

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