Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 814 (2005) 115–125
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Chromatographic evaluation of the toxicity in fish of pesticides

José María Bermúdez-Saldaña, Laura Escuder-Gilabert, María José Medina-Hernández, Rosa María Villanueva-Camañas, Salvador Sagrado*

Departamento de Química Analítica, Facultat de Farmacia, Universitat de València, C/Vicente Andrés Estellés s/n E-46100, Burjassot, Valencia, Spain

Received 27 July 2004; accepted 4 October 2004
Available online 11 November 2004

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 toxicity in fish of pesticides (acute toxicity levels as pLC_{50}) is proposed. A heterogeneous data set of 85 pesticides from six chemical families with available experimental fish toxicity data (ECOTOX database from U.S. Environmental Protection Agency (EPA)) was used. For pesticides exhibiting non-polar narcosis mechanism in fish (non-specific toxicity), more reliable models and precise pLC_{50} estimations are obtained from log *k* (quantitative retention–activity relationships, QRAR) than from log *P* (quantitative structure-activity relationships, QSAR) or ECOSAR (ECOSAR program from U.S. EPA).

Keywords: Fish toxicity; Non-polar narcosis mechanism of action; Pesticides; Biopartitioning micellar chromatography

1. Introduction

Agricultural development has led a parallel growth in the use of chemical agents for plague controls, which are known as pesticides. These compounds are released into the environment and due to their physico-chemical properties, such as water solubility, vapor pressure or partition coefficients between organic matter (in soil or sediment) and water, they can disperse in various environmental media provoking serious health problems [1]. Their wide and extensive use provokes, according to the World Health Organization, about 25 millions of poisoning cases and 20.000 involuntary deaths, mainly in developing countries. Therefore, there is a need of evaluating the potential hazard of pesticides for risk assessment.

Aquatic toxicity tests, for instance lethal concentration (i.e. LC_{50}) in fish, are current methods applied by the European community for ecotoxicity estimation [2]. The experimental determinations are difficult, time-consuming, and expensive. Several experimental factors have to be controlled or selected, for instance, the oxygen and compound concentrations, pH, temperature, photo periods, animal characteristics, procedure (i.e. static, semistatic or dynamic assay), exposure time (i.e. 96 h for fish tests), etc., which contribute to a considerable inter-laboratory variability on the experimental reported values. Now-a-days, the use of alternative (in vitro) methods is growing due to several reasons, such as ethical, economic, logistical, etc. [3].

Toxicity have been divided into three broad categories: non-covalent or non-specific narcosis, covalent reactivity as a result of electro(nucleo)philic interactions with some biological macromolecule, and receptor-mediated functional toxicity where interactions with receptors are typically a non-covalent, 'lock-and-key-type' interface. Narcosis mode of action is the result of the accumulation of compounds in the cell membranes, which disturbs their normal function by disruption of hydrophobic interactions within the membrane. It is considered to be dependent on the hydrophobicity of chemicals (i.e. the octanol-water partition coefficient or log *P*). This mode of action includes several mechanisms of action, such as non-polar narcosis or polar narcosis among others [4]. The

* Corresponding author. Tel.: +34 963544878; fax: +34 963544953.
E-mail address: sagrado@uv.es (S. Sagrado).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.10.007

log *P*-toxicity relationships for non-polar narcosis are known as baseline toxicity [5,6], since chemicals with different mode or mechanism of action (i.e polar narcosis) show equal or higher toxicity than the predicted by these relationships. On the other hand, specific toxicity is due to the disruption of the function of a defined receptor site in the cell. Such compounds tend to show toxicity values between 10 and 10 000 times higher than those predicted by the baseline toxicity [7].

The log *P*-toxicity relationships falls into the so-called qualitative or quantitative structure–activity relationships (SAR or QSAR), and they are used as an alternative to in vivo assays. SARs have been used by the U.S. Environmental Protection Agency (EPA), since 1981, to predict the aquatic toxicity of new industrial chemicals in the absence of test data. Today, EPA provides the ECOSAR program, a set of QSARs to estimate different parameters with ecotoxicological interest as LC_{50} in fish (96-h) [8]. In QSAR models, besides log *P*, other descriptors (i.e. electronic, steric, etc.) have been used as predictor variables [9].

Chromatographic separations have found a wide application in the environmental field. For instance, they have become a fundamental tool for multiresidue (qualitative and quantitative) analysis of water samples, containing pesticides [10]. On the other hand, the use of the retention of compounds in reversed-phase liquid chromatography (RPLC), under adequate experimental conditions, has been also proposed as an alternative to in vivo tests. The models derived from the use of retention data (i.e. log *k*) are called quantitative retention-activity relationships (QRAR) [11]. Könemann compared the relationships between fish toxicity and log *P*, log *k* (using C_{18} stationary phases and hydro-organic mobile phases with methanol), water solubility and molecular connectivity indices, showing the best correlations for log *P* and log *k* [5]. Warne et al. determined the suitability of different stationary phases to describe non-specific toxicity of compounds to marine bacteria, showing the best retention-toxicity linear regression for the combination between non-polar (C_{18}) and polar (amino) stationary phases connected in series [12]. Dai et al. [13] combined the extrapolated retention factor to 100% water, log k_w, and other quantum chemical parameters to model the toxicity of benzaldehyde compounds to Daphnia Magna.

Opposite to octanol-water and conventional RPLC systems, micelles have proven to be more adequate chemical models for biomembranes mainly due to their amphiphilic and anisotropic properties [11,14,15]. Micellar liquid chromatography is a mode of reversed-phase liquid chromatography, which uses surfactant solutions above the critical micellar concentration as mobile phases. The use of retention data obtained in a chromatographic system constituted by polioxyethylene(23)lauryl ether (Brij35) solutions as micellar mobile phases and C_{18} reversed stationary phase, under the adequate experimental conditions, has proven to be very useful for describing the biological activities of compounds [16,17]. This approach, called biopartitioning micellar chromatography (BMC) [18], emulates the partitioning of chemicals in biomembranes. The retention in BMC of organic pollutants has been related with ecotoxicological parameters estimated by ECOSAR [19,20].

The aim of the present study was to investigate the use of the BMC chromatographic retention (log *k*) as an in vitro approach for the ecotoxicity evaluation of pesticides (acute toxicity levels in fish). A heterogeneous data set of 85 pesticides with available experimental toxicity data (ECOTOX database from EPA) was used. The role of pesticides mechanism of action was studied. Finally, the toxicity levels estimated by means of log *k* (QRAR), log *P* (QSAR) and ECOSAR (from EPA) approaches were compared.

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 was employed to obtained retention data. Data acquisition and processing were performed by means of a HP Vectra XM computer (Amsterdam, The Netherlands) equipped with a HP-Chemstation software (A.07.01 [682] ©HP 1999). Two Kromasil C_{18} columns (5 μm, 150 mm × 4.6 mm i.d.; Scharlab S.L., Barcelona, Spain) and (5 μm, 50 mm × 4.6 mm i.d.; Scharlab S.L.) were used (for less and more hydrophobic compounds, respectively). 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 S.L). 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, WI, USA), DC, MCPA, 2,4-D, MCPB, 2,4,5-T, trichlorfon, dimethoate, methidathion, malathion, mecarbam, pirimiphos-methyl, chlorpyrifos-methyl, diazinon, fenthion, chlorpyrifos, pir-

imicarb, 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 S.L.), 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. Linear discriminant analysis (LDA) was implemented via STATGRAPHICS 5.1 (Demo version). The default forward variable selection criteria (F-to-enter and F-to-remove equal to 4) were selected in the stepwise introduction of variables into the model. Prior probabilities were set proportional to group size (based on the initially assigned mechanism of action of pesticides). All other calculations were performed using routines developed in MATLAB 5.3 (Matlab Ver. 5.3.0.10183 (R11), ©The Mathwoks Inc., Natick, MA).

ECOSAR software (ECOWIN version 0.99f) was used for LC_{50} in fish (96-hours) estimations and KOWWIN software (version 1.66) was used for $\log P$ estimations. These programmes are integrated in the EPI Suite software (developed by Syracuse Research Corporation for the U.S Enviromental Protection Agency). Structural parameters were calculated using the ACD Labs™ (version 5.12, Advanced Chemistry Development Inc. Toronto) software [21].

2.4. Retention factor estimations

The retention factor (k) of pesticides was estimated according to an approach described elsewhere [22]:

$$k = [(t_R/t_{R(REF)})(1 + k_{REF})] - 1, \quad (1)$$

where t_R is the experimental retention time of the pesticide assayed and $t_{R(REF)}$ 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 [23]). For instance, this approach reduces the impact of changing the column and mobile phase flow rate, among other experimental factors, on the k estimations [22].

2.5. Data

The 85 pesticides data set is shown in Table 1. The variables (coded name) used were: FAM (indicates the chemical family of pesticides): (1) organophosphorous, (2) carbamates, (3) triazines, (4) organochlorines, (5) phenoxy-acids, (6) phenylureas. ECL (indicates the chemical class of pesticides according to ECOSAR software): (1) neutral organics, (2) esterphosphates, (3) esters, (4) neutral organic acids, (5) aromatic amines, (6) vinyl allyl halides, (7) benzyl halides. MEC (indicates the initial assigned mechanism of toxic action): (1) non-polar narcosis (non-specific toxicity), (2) unknown mechanism, (3) acetylcholinesterase (AChE) inhibitors (specific toxicity).

The variables $\log k2$, $\log k4$ and $\log k6$ are the logarithm of the retention factors obtained with 0.02, 0.04 and 0.06 M Brij35 mobile phases, respectively. pLC_{50} represents the minus logarithm of the LC_{50} ($mg\,L^{-1}$) in fish (96 h-test); $E - pLC$ refers to ECOSAR (from U.S. EPA) pLC_{50} estimations, whereas, pLC (r) and pLC(b) correspond to the pLC_{50} median values (experimental data found in ECOTOX database from U.S. EPA [24]) for rainbow trout and bluegill, respectively.

Table 2 shows some available pesticide descriptors: $\log P$ and $E - \log P$ are the experimental and estimated by KOWWIN values of the octanol-water partition coefficient, respectively; log Sol (the logarithm of water solubility in $mg\,L^{-1}$), MW (the molecular weight), MP (the melting point in °C), log VP (the logarithm of vapor pressure in mmHg) and Henry (the Henry law constant in $atm\,m^3\,mol^{-1}$), were obtained from PhysProp database [25] (mainly providing experimental values but also some estimated ones, set in bold-face case in Table 2); MR (molar refractivity in cm^3), MV (molar volume in cm^3), Par (parachor in cm^3), Pol (polarizability in cm^3), ST (surface tension in dyne cm^{-1}), Den (density in $g\,cm^{-3}$) and IR (index of refraction) were estimated using ACD Labs™ software [21].

The $\log k2$, $\log k4$ and $\log k6$ values for pesticides with $E - \log P < 2.5$ were obtained using the 150 mm-length column. For the rest of pesticides the 50 mm-length column was used.

3. Results and discussion

3.1. Data reliability

QSAR models quality depends on the reliability of data (i.e. uncertainty in toxicological and physico-chemical and/or structural data) [17,26]. Unfortunately, this point is not taken into account in most studies; however, it deserves more attention, since it could affect the decision-making in toxicity assessment of pesticides. Fig. 1 compares the experimen-

Table 1
Categorical, retention and fish toxicity data of pesticides

N	CAS	Compounds	FAM	ECL	MEC	log *k*2	log *k*4	log *k*6	*E* − pLC	pLC(*r*)	pLC(*b*)
1	2157-98-4	Monocrotophos	1	2	3	0.321	0.207	0.255	−2.957	–	–
2	10265-92-6	Methamidophos	1	1	3	0.117	0.007	0.095	−4.768	−1.3979	−1.6532
3	52-68-6	Trichlorfon	1	1	3	0.412	0.722	0.296	−4.424	0.0758	−0.4624
4	60-51-5	Dimethoate	1	2	3	1.068	0.941	0.860	−2.146	−0.8337	−0.7782
5	950-37-8	Methidathion	1	2	3	1.993	1.748	1.546	−1.593	1.8539	2.0458
6	121-75-5	Malathion	1	2	3	2.153	1.893	1.686	−1.264	0.9547	1.0482
7	2595-54-2	Mecarbam	1	2	3	2.131	1.885	1.693	−1.262	–	–
8	732-11-6	Phosmet	1	2	3	1.979	1.714	1.503	−1.148	0.2764	0.3768
9	298-00-0	Parathion-methyl	1	2	3	2.083	1.817	1.604	−0.927	−0.5077	−0.5139
10	29232-93-7	Pirimiphos-methyl	1	2	3	2.384	2.116	1.894	−0.634	0.1646	−0.4564
11	2642-71-9	Azinphos-ethyl	1	2	3	2.156	1.887	1.672	−0.651	1.6990	2.9586
12	5598-13-0	Chlorpyrifos-methyl	1	2	3	2.353	2.085	1.863	−0.534	0.9208	0.0555
13	333-41-5	Diazinon	1	2	3	2.379	2.112	1.896	−0.415	0.1972	0.7696
14	55-38-9	Fenthion	1	2	3	2.234	1.965	1.745	−0.262	0.0757	−0.1852
15	13067-93-1	Cyanofenphos	1	1	3	2.307	2.037	1.813	−0.284	–	–
16	2310-17-0	Phosalone	1	2	3	2.448	2.179	1.949	−0.275	0.8239	1.0000
17	56-72-4	Coumaphos	1	2	3	2.282	2.014	1.790	−0.176	−0.0627	0.7447
18	2921-88-2	Chlorpyrifos	1	2	3	2.569	2.300	2.073	−0.062	1.8468	2.5229
19	57018-04-9	Tolclofos-methyl	1	2	3	2.305	2.037	1.818	0.060	–	–
20	23135-22-0	Oxamyl	2	1	3	0.326	0.169	0.258	−5.220	−0.6477	−0.8068
21	1646-88-4	Aldoxycarb	2	1	3	0.296	0.191	0.239	−4.727	−1.6232	−1.7243
22	16752-77-5	Methomyl	2	1	3	0.511	0.409	0.414	−3.387	−0.1761	0.0655
23	23103-98-2	Pirimicarb	2	3	3	1.553	1.354	1.248	−1.878	−2.0934	−1.8162
24	114-26-1	Propoxur	2	3	3	1.446	1.280	1.163	−1.554	−0.9138	−0.7924
25	17804-35-2	Benomyl	2	3	3	1.345	1.149	1.009	−1.514	0.6383	−0.0792
26	1563-66-2	Carbofuran	2	3	3	1.511	1.328	1.196	−1.364	0.4202	0.6198
27	63-25-2	Carbaryl	2	3	3	1.735	1.493	1.297	−1.296	−0.1537	−0.8299
28	2212-67-1	Molinate	2	1	3	2.050	1.814	1.644	−1.287	−0.9191	−1.2200
29	1114-71-2	Pebulate	2	1	3	2.399	2.130	1.925	−0.759	−0.8692	−0.8976
30	82560-54-1	Benfuracarb	2	3	3	2.516	2.257	2.024	−0.691	–	–
31	122-34-9	Simazine	3	1	1	1.609	1.394	1.239	−1.799	−1.8482	−2.0000
32	21725-46-2	Cyanazine	3	1	1	1.647	1.415	1.244	−1.772	−0.9542	−1.3075
33	1014-69-3	Desmetryn	3	1	1	1.721	1.566	1.340	−1.428	–	–
34	841-06-5	Methoprotryn	3	1	1	1.849	1.674	1.445	−1.326	−0.9031	−1.1903
35	4658-28-0	Aziprotryne	3	1	1	2.043	1.790	1.594	−1.029	–	–
36	5915-41-3	Terbuthylazine	3	1	1	1.995	1.736	1.536	−1.037	−0.5971	−0.8751
37	834-12-8	Ametryne	3	1	1	1.889	1.698	1.470	−0.986	−0.5315	−0.6128
38	1610-18-0	Prometon	3	1	1	1.837	1.599	1.432	−0.747	−1.2041	−1.5536
39	7287-19-6	Prometryn	3	1	1	2.077	1.815	1.613	−0.626	−0.6599	−1.0000
40	886-50-0	Terbutryne	3	1	1	2.084	1.845	1.622	−0.589	−0.2553	−0.4346
41	4147-51-7	Dipropetryn	3	1	1	2.207	1.942	1.731	−0.190	-	-
42	108-90-7	Chlorobenzene	4	1	1	2.060	1.820	1.632	−1.320	−0.7724	−1.0367
43	99-30-9	Dicloran	4	5	1	1.931	1.668	1.457	−1.232	−0.2041	−0.8451
44	95-50-1	1,2-Dichlorobenzene	4	1	1	2.126	1.867	1.662	−0.834	−0.1987	−1.0898
45	106-46-7	1,4-Dichlorobenzene	4	1	1	2.240	1.982	1.780	−0.834	−0.0492	−0.7198
46	541-73-1	1,3-Dichlorobenzene	4	1	1	2.195	1.952	1.746	−0.834	–	−0.6990
47	33213-65-9	β-Endosulfan	4	6	1	2.424	2.176	1.923	0.057	3.0889	2.4815
48	120-82-1	1,2,4-Trichlorobenzene	4	1	1	2.311	2.046	1.835	−0.314	−0.1847	−0.5057
49	108-70-3	1,3,5-Trichlorobenzene	4	1	1	2.392	2.128	1.919	−0.314	–	–
50	87-61-6	1,2,3-Trichlorobenzene	4	1	1	2.206	1.944	1.730	−0.314	–	–
51	510-15-6	Chlorbenzylate	4	3	1	2.401	2.130	1.903	−0.627	0.1644	–
52	5836-10-2	Chlorpropylate	4	3	1	2.509	2.239	2.009	−0.421	–	–
53	608-93-5	Pentachlorobenzene	4	1	1	2.504	2.236	2.021	0.759	–	0.6021
54	118-74-1	Hexachlorobenzene	4	1	1	2.623	2.354	2.137	1.301	–	−1.0792
55	3547-04-4	DDE	4	1	1	2.769	2.497	2.262	0.963	1.3010	1.3010
56	72-54-8	DDD	4	7	1	2.693	2.420	2.184	0.873	1.1549	1.3768
57	115-32-2	Dicofol	4	7	1	2.429	2.413	2.175	0.801	0.7922	0.2840
58	940-31-8	2-PPA	5	4	2	0.175	−0.026	0.090	−3.326	–	–
59	122-88-3	4-CPA	5	4	2	1.218	0.863	0.836	−3.169	–	−2.2553
60	1918-00-9	DC	5	4	2	0.346	0.145	0.246	−3.083	−2.1139	−1.9151
61	101-10-0	3-CPPA	5	4	2	0.792	0.527	0.562	−2.806	–	–

Table 1 (*Continued*)

N	CAS	Compounds	FAM	ECL	MEC	log *k*2	log *k*4	log *k*6	*E* − pLC	pLC(*r*)	pLC(*b*)
62	3307-39-9	4-CPPA	5	4	2	0.812	0.544	0.573	−2.806	–	–
63	94-74-6	MCPA	5	4	2	1.207	0.844	0.816	−2.684	−1.9590	−1.4934
64	94-75-7	2,4-D	5	4	2	1.211	0.855	0.833	−2.632	−1.2765	−2.3376
65	93-65-2	MCPP	5	4	2	1.291	0.904	0.876	−2.318	−1.0000	–
66	120-36-5	2,4-DCPPA	5	4	2	1.317	0.936	0.908	−2.273	−0.5745	−0.3802
67	93-76-5	2,4,5-T	5	4	2	1.482	1.067	1.002	−2.093	−0.0578	–
68	94-81-5	MCPB	5	4	2	1.702	1.251	1.135	−1.819	1.1549	−0.5185
69	93-72-1	2,4,5-TCPPA	5	4	2	1.556	1.135	1.062	−1.721	−1.2355	−1.0426
70	113158-40-0	Fenoxaprop-P	5	4	2	1.552	1.084	1.027	−1.354	–	–
71	101-42-8	Fenuron	6	1	1	1.012	0.886	0.823	−2.668	−2.3096	–
72	150-68-5	Monuron	6	1	1	1.591	1.372	1.194	−2.140	–	−1.5190
73	19937-59-8	Metoxuron	6	1	1	1.435	1.232	1.073	−2.126	–	–
74	1746-81-2	Monolinuron	6	1	1	1.769	1.535	1.340	−1.957	–	–
75	2164-17-2	Fluometuron	6	1	1	1.789	1.554	1.352	−1.907	−1.3838	−1.6812
76	15545-48-9	Chlorotoluron	6	1	1	1.736	1.486	1.294	−1.653	−1.5441	−1.6990
77	18691-97-9	Methabenzthiazuron	6	1	1	1.748	1.490	1.292	−1.604	–	–
78	330-54-1	Diuron	6	1	1	1.800	1.543	1.334	−1.608	−0.8633	−0.9164
79	34123-59-6	Isoproturon	6	1	1	1.770	1.531	1.349	−1.395	–	–
80	330-55-2	Linuron	6	1	1	1.920	1.653	1.443	−1.411	−0.8460	−0.9823
81	13360-45-7	Chlorbromuron	6	1	1	1.936	1.672	1.456	−1.257	−0.1523	−0.6990
82	1982-47-4	Chloroxuron	6	1	1	1.955	1.689	1.473	−0.378	−0.8167	−1.4472
83	555-37-3	Neburon	6	1	1	2.218	1.957	1.725	−0.289	–	–
84	3766-60-7	Buturon	6	1	1	2.033	1.776	1.558	−1.624	–	–
85	25366-23-8	Thiazafluron	6	1	1	1.735	1.532	1.343	−2.392	–	–

tal pLC_{50} values (median and interquartile range) reported for rainbow trout and bluegill by means of a pLC(*r*) versus pLC(*b*) relationship of each compound. As can be observed, there is a relatively high correlation ($r^2 = 0.84$) of the data. However, some pesticides show different pLC_{50} values depending on the target organism (i.e >1-pLC_{50} units for compounds $N = 11, 64, 68$), being unfeasible to decide whether there is different inter-organisms sensitivity or an error in the experimental pLC_{50} values. In addition, some compounds show interquartile ranges larger than one pLC_{50} unit, which evidences the uncertainty associated with experimental pLC_{50} data.

Table 2
Descriptors of pesticides

N	log *P*	*E* − log *P*	log Sol	MW	MP	log VP	Henry	MR	MV	Par	Pol	ST	Den	IR
1	−0.20	−1.31	6.000	223.17	55	−5.66	**1.67E-10**	50.11	186.6	458.1	19.86	36.2	1.195	1.449
2	−0.80	−0.93	6.000	141.13	46	−4.45	**3.16E-08**	31.46	109.7	283.1	12.47	44.4	1.286	1.485
3	0.51	−0.28	5.079	257.44	77	−5.11	1.70E-11	46.95	163.5	427.2	18.61	46.6	1.574	1.486
4	0.78	0.28	4.398	229.25	52	−5.08	1.05E-10	54.45	175.7	467.6	21.58	50.1	1.304	1.532
5	2.20	1.58	2.272	302.32	39	−5.47	7.17E-09	68.92	188.5	525.7	27.32	60.4	1.6	1.651
6	2.37	2.29	2.155	330.35	2.8	−5.47	4.89E-09	77.5	259.6	680.4	30.72	47.1	1.272	1.508
7	–	2.29	3.000	329.37	25	−5.49	1.53E-08	79.52	260.1	689	31.52	49.2	1.266	1.523
8	2.78	2.48	1.387	317.32	72	−6.31	8.38E-09	77.22	215.3	615.2	30.61	66.5	1.473	1.636
9	2.86	2.75	1.576	263.21	35.5	−5.46	1.00E-07	61.73	186.5	512.1	24.47	56.8	1.411	1.576
10	4.20	3.44	0.934	305.33	15	−4.82	7.01E-07	79.77	248.4	664.1	31.62	51.1	1.229	1.555
11	3.40	3.52	1.021	345.37	53	−5.62	9.95E-08	88.06	241.1	661.2	34.91	56.4	1.43	1.65
12	4.31	3.69	0.678	322.53	43	−4.38	3.75E-06	67.96	203.7	558.4	26.94	56.4	1.582	1.581
13	3.81	3.86	1.602	304.35	25	−4.05	1.13E-07	79.64	260.4	677.2	31.57	45.6	1.168	1.523
14	4.09	4.09	0.875	278.32	7.5	−4.98	1.46E-06	72.23	221.8	586.5	28.63	48.8	1.25	1.564
15	4.29	4.20	−0.222	303.32	83	−5.92	3.49E-07	82.5	239.2	658.7	32.7	57.4	1.26	1.606
16	4.38	4.29	0.484	367.80	46	−6.92	**6.61E-08**	88.24	254.8	707.6	34.98	59.4	1.443	1.608
17	4.13	4.47	0.176	362.77	93	−7.01	3.09E-08	86.84	261.8	709.5	34.42	53.9	1.38	1.577
18	4.96	4.66	0.049	350.59	42	−4.69	2.93E-06	77.23	236.7	637.9	30.61	52.7	1.48	1.565
19	4.56	4.77	0.041	301.13	79	−3.37	1.54E-04	69.8	214.8	566	27.67	48.1	1.401	1.563
20	−0.48	−1.20	5.447	219.26	101	−3.64	2.37E-10	55.03	177.6	448.1	21.81	40.5	1.23	1.531
21	−0.57	−0.67	4.000	222.26	141	−4.05	3.37E-09	52.69	180.6	454.9	20.89	40.1	1.23	1.495
22	0.60	0.61	4.763	162.21	78	−5.27	1.97E-11	41.18	137.9	338.6	16.32	36.3	1.17	1.508
23	1.70	1.40	3.431	238.29	90.5	−5.14	3.48E-09	66.09	207.8	539.8	26.2	45.5	1.146	1.548
24	1.52	1.90	3.270	209.25	87	−5.01	1.43E-09	57.09	193.2	470.3	22.63	35	1.082	1.502

Table 2 (*Continued*)

N	log *P*	*E* − log *P*	log Sol	MW	MP	log VP	Henry	MR	MV	Par	Pol	ST	Den	IR
25	2.12	2.24	0.580	290.32	140	−8.43	4.93E-12	77.8	225.2	594.1	30.84	48.4	1.28	1.607
26	2.32	2.30	2.505	221.26	151	−5.31	3.09E-09	59.67	194.4	490.5	23.65	40.5	1.137	1.525
27	2.36	2.35	2.041	201.23	145	−5.87	4.36E-09	59.03	169.9	440.5	23.4	45.1	1.183	1.611
28	3.21	2.91	2.987	187.30	25	−2.25	4.10E-06	53.07	176.9	444.8	21.03	39.9	1.058	1.511
29	3.83	3.51	2.000	203.35	25	−1.05	2.37E-04	60.12	211.9	511.4	23.83	33.8	0.959	1.479
30	4.30	4.06	0.903	410.53	25	−6.70	1.35E-08	110.31	349.9	905	43.73	44.7	1.172	1.542
31	2.18	2.40	0.792	201.66	226	−7.66	9.42E-10	53.9	152.9	422.9	21.37	58.4	1.318	1.622
32	2.22	2.51	2.230	240.70	168	−6.86	2.96E-12	63.05	179.4	505.8	24.99	63.1	1.341	1.62
33	2.38	2.82	2.763	213.30	85	−6.00	4.83E-10	58.57	180.2	493.8	23.22	56.4	1.18	1.563
34	2.82	3.04	2.505	271.38	**155.3**	−6.55	3.18E-10	74.21	235.4	634.4	29.41	52.7	1.15	1.543
35	3.00	3.27	1.740	225.27	**217.88**	−9.91	1.12E-12	–	–	–	–	–	–	–
36	3.22	3.27	0.929	229.71	178	−5.95	3.72E-08	63.13	186	497.5	25.02	51.1	1.234	1.594
37	2.98	3.32	2.320	227.33	88	−5.56	2.39E-09	63.2	196.5	533.9	25.05	54.4	1.15	1.556
38	2.99	3.57	2.875	225.30	91.5	−5.64	3.17E-09	64.87	198.7	518	25.71	46.1	1.133	1.566
39	3.51	3.73	1.519	241.36	119	−5.70	1.32E-08	67.81	213.5	571.9	26.88	51.4	1.13	1.547
40	3.74	3.77	1.398	241.36	104	−5.68	1.15E-08	67.86	212.7	572.3	26.9	52.3	1.13	1.55
41	3.81	4.22	1.204	255.38	105	−5.80	3.36E-08	72.44	229.8	612	28.71	50.2	1.11	1.542
42	2.84	2.64	2.697	112.56	−45.2	1.08	3.11E-03	31.14	101.3	243.1	12.34	33	1.11	1.526
43	2.80	2.76	0.845	207.02	191	−5.92	4.67E-08	46.82	127.4	360.3	18.56	63.8	1.624	1.655
44	3.43	3.28	2.193	147.00	−16.7	0.17	1.92E-03	36.04	113.3	279	14.28	36.7	1.297	1.548
45	3.44	3.29	1.910	147.00	52.7	0.24	2.41E-03	36.04	113.3	279	14.28	36.7	1.297	1.548
46	3.53	3.30	2.097	147.00	−24.8	0.33	2.63E-03	36.04	113.3	279	14.28	36.7	1.297	1.548
47	3.83	3.50	−0.488	406.92	109	−6.22	6.50E-05	78.38	208.7	614.1	31.07	74.9	1.94	1.674
48	4.02	3.93	1.690	181.45	17	−0.34	1.42E-03	40.93	125.2	314.8	16.22	39.9	1.448	1.567
49	4.19	3.93	0.779	181.45	63.5	−0.34	1.89E-03	40.93	125.2	314.8	16.22	39.9	1.448	1.567
50	4.05	3.93	1.255	181.45	53.5	−0.68	1.25E-03	40.93	125.2	314.8	16.22	39.9	1.448	1.567
51	4.74	3.99	1.114	325.19	37	−5.66	7.24E-08	82.13	244.1	642.7	32.56	48	1.332	1.587
52	–	4.41	1.000	339.22	73	−6.74	8.03E-09	86.72	260.9	679.9	34.38	46	1.299	1.578
53	5.17	5.22	−0.080	250.34	86	−3.00	7.03E-04	50.72	149.1	386.6	20.11	45.1	1.678	1.595
54	5.73	5.86	−2.208	284.78	231.8	−4.74	1.70E-03	55.62	161.1	422.4	22.05	47.2	1.767	1.606
55	–	5.44	−0.277	251.16	56	−3.46	**2.16E-04**	69.99	209.9	528.5	27.74	40.1	1.195	1.581
56	6.02	5.87	−1.046	320.05	109.5	−5.87	6.60E-06	79.65	233.1	602.7	31.57	44.6	1.372	1.599
57	5.02	5.81	−0.097	370.49	77.5	−6.40	2.42E-07	85.61	241.8	648.8	33.93	51.8	1.532	1.626
58	–	1.75	3.711	166.18	**74.35**	−2.94	**4.94E-08**	43.72	141.4	362.6	17.33	43.2	1.174	1.53
59	2.25	1.97	2.981	186.60	164	−4.31	**7.88E-09**	44.02	136.5	361.3	17.45	49.1	1.366	1.558
60	2.21	2.14	3.920	221.04	115	−4.47	2.18E-09	49.65	149.8	397.8	19.68	49.6	1.474	1.576
61	–	2.39	3.079	200.62	113	−3.88	**3.55E-08**	48.61	153.4	398.5	19.27	45.5	1.307	1.546
62	2.31	2.39	3.167	200.62	**90.51**	−3.64	**5.24E-08**	48.61	153.4	398.5	19.27	45.5	1.307	1.546
63	3.25	2.52	2.799	200.62	120	−5.23	1.33E-09	48.84	152.7	399	19.36	46.5	1.313	1.552
64	2.81	2.62	2.831	221.04	140.5	−4.08	3.54E-08	48.91	148.4	397.2	19.39	51.2	1.488	1.572
65	3.13	2.94	2.934	214.65	95	−5.52	8.96E-10	53.44	169.6	436.2	21.18	43.6	1.265	1.542
66	3.43	3.03	2.544	235.07	122	−7.12	**1.26E-07**	53.51	165.3	434.4	21.21	47.6	1.421	1.56
67	3.31	3.26	2.444	255.49	153	−4.43	**2.89E-08**	53.81	160.4	433.1	21.33	53.1	1.592	1.585
68	–	3.50	1.681	228.68	100	−6.36	2.71E-09	58.11	185.8	478.6	23.03	44	1.23	1.537
69	3.80	3.68	1.851	269.51	181.6	−5.59	**3.52E-08**	58.4	177.3	470.2	23.15	49.5	1.52	1.572
70	–	4.17	2.431	333.73	158	−5.87	2.20E-09	83.29	234.1	641.6	33.01	56.4	1.425	1.629
71	0.98	1.38	3.605	164.21	133.5	−4.43	2.01E-09	48.87	146.2	376.4	19.37	43.8	1.122	1.582
72	1.94	2.03	2.362	198.65	170.5	−6.30	5.72E-10	53.77	158.2	412.3	21.31	46.1	1.255	1.595
73	1.64	2.11	2.831	228.68	126.5	−4.49	1.43E-08	60.45	182.2	469	23.96	43.8	1.254	1.577
74	2.30	2.26	2.968	214.65	81.5	−3.82	4.60E-08	55.51	164.5	432	22	47.4	1.304	1.589
75	2.42	2.36	2.041	232.21	164	−6.03	1.80E-09	53.85	179.7	433.6	21.35	33.8	1.291	1.51
76	2.41	2.58	1.845	212.68	148.1	−7.44	1.44E-10	58.59	174.5	449.9	23.23	44.2	1.218	1.586
77	2.64	2.65	1.771	221.28	120	−6.95	5.58E-10	63.14	166.6	463.2	25.03	59.6	1.327	1.682
78	2.68	2.67	1.623	233.10	158	−7.16	5.04E-10	58.66	170.1	448.2	23.25	48.1	1.369	1.605
79	2.84	2.87	1.813	206.29	158	−7.61	1.12E-10	63.06	196.3	491.5	25	39.2	1.05	1.555
80	3.20	2.92	1.875	249.10	93	−5.84	6.25E-09	60.41	176.5	467.8	23.95	49.3	1.41	1.6
81	3.09	3.15	1.544	293.55	96	−6.40	4.38E-09	63.2	180.7	482.5	25.05	50.7	1.623	1.616
82	3.70	4.08	0.568	290.75	151	−8.41	4.03E-10	80.21	228.8	603.4	31.8	48.3	1.27	1.618
83	4.10	4.15	0.681	275.18	102	−5.56	**1.87E-07**	72.56	219.6	567.5	28.76	44.5	1.252	1.574
84	3.00	2.66	1.477	236.70	**130.53**	−5.34	**1.85E-08**	65.45	191.8	508	25.95	49.1	1.233	1.597
85	1.85	0.83	3.322	240.20	136.5	−5.44	**2.28E-11**	48.37	159.3	410.7	19.17	44.2	1.507	1.519

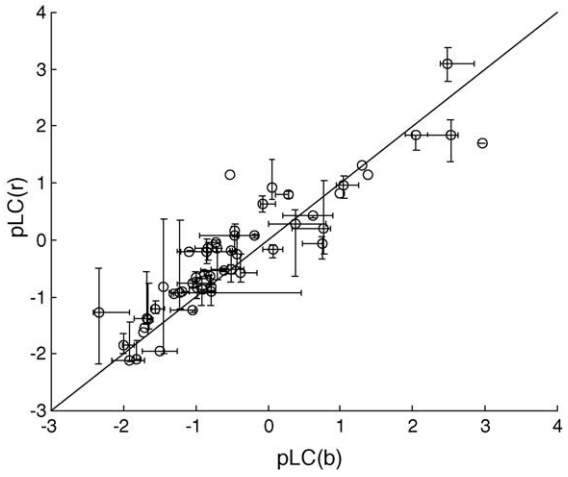

Fig. 1. Correlation between the pLC_{50} median values for rainbow trout and bluegill, including the available interquartile ranges. The pLC(r) = pLC(b) reference line is also included.

log P, which is the dominating independent parameter in the field of aquatic toxicity, can also contribute to the uncertainty of the QSAR models. Experimental values are difficult to obtain and become unreliable if log $P > 4$ [5]. In addition, it is difficult to find these values for all compounds of interest. There has been increasing interest in using log P values estimated using computer algorithms, however, assignment of probable error to the calculation of log P is very difficult [27]. Table 2 allows the comparison of the variables log P (experimental) and $E -$ log P (estimated by KOWWIN software). The $E -$ log P versus log P linear equation found (intercept = -0.22 ± 0.19; slope = 1.04 ± 0.06; where intervals are the confidence limits at 95% confidence level; $n = 78$; $r^2 = 0.94$, $F = 1256$; $p < 0.05$), suggests that $E -$ log P values can be used in this case, which avoids the need of excluding those pesticides for whose experimental log P values are not available. However, 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.

Alternatively, 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 [20]. 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 [22].

3.2. Mechanisms of action

A current opinion is that high quality QSARs can only be developed for compounds with a common toxicity mechanism [28]. Also, studies in the literature have shown that it is more appropriate to combine the compounds by their toxicity mechanisms instead of chemical classes [29,30]. However, correctly determining the mechanism of a compound is not always straightforward [31,32], and therefore, errors in mechanism assignation would be transferred to the QSARs toxicity estimation [33].

Initially, we assigned the toxicity mechanism (variable MEC in Table 1) on the basis of the information reported in the bibliography, so, non-polar narcosis (MEC = 1) was assigned to the family of organochlorines (FAM = 4) [6]. The code MEC = 1 was set to indicated a relatively low toxicity level. AChE inhibitors (MEC = 3) was assigned to organophosphorous (FAM = 1) and carbamates (FAM = 2) [3]. The code MEC = 3 was set to indicated a relatively high toxicity level. For triazines (FAM = 3) and phenylureas (FAM = 6) no bibliographic information about their mechanism of action was found. ECOSAR includes the compounds of these two families into the 'neutral organic class' (ECL = 1), which corresponds to non-polar narcosis mechanism. Therefore, a MEC = 1 was assigned for these pesticides. Finally, no bibliographic information about the mechanism of action of phenoxyacids (FAM = 5) was found. Some of these ionic compounds could fit the characteristics of respiratory uncouplers (i.e acidity constant between 4 and 8 and good delocalization of the charge). For instance, this mechanism was described for three fenoxiacids in rat liver [34–36]. On the other hand, it could be possible to consider a narcosis mode of action. In these conditions, the toxicity mechanism in fish is unknown but it is reasonable to presume a different mechanism from those previously mentioned with an intermediate level of toxicity (MEC = 2).

Regardless the criteria used for assigning toxicity mechanism to a compound, it is important to statistically contrast it and to identify possible misclassification of compounds, at least to have more elements for decision-making. Linear discriminant analysis (LDA) has been used frequently to perform these tasks [33]. The retention variables in Table 1 (log $k2$, log $k4$, log $k6$) and all the molecular descriptors in Table 2 (except log P), totalling 16 variables, were submitted to LDA, together with the MEC variable as discriminant factor. Since, some variables in Table 2 are correlated, a variable selection step is convenient in order to reduce redundant information and select the variables with the most discriminant capacity. The forward variable selection step selected (in order) the variables: log $k4$, $E -$ log P, log $k2$, MV, MP, log Sol, Den and Pol, in the final LDA model. Fig. 2 shows the dis-

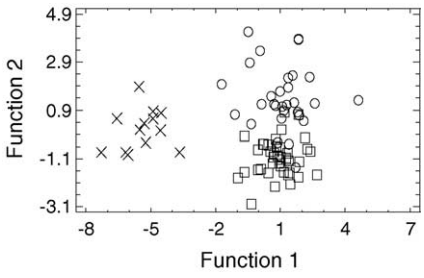

Fig. 2. Plot of discriminant functions 1 and 2. (□) MEC = 1; (×) MEC = 2; (◯) MEC = 3.

Table 3
Summary of the classification results

	Group size	MEC = 1	MEC = 2	MEC = 3
Number of MEC = 1 pesticides classified as	41	39 (95.12%)	0	2 (4.88%)
Number of MEC = 2 pesticides classified as	13	0	13 (100%)	0
Number of MEC = 3 pesticides classified as	30	4 (13.33%)	0	26 (86.67%)

Percent of cases correctly classified: 92.86%.

criminant function plot obtained. As can be observed, function 1 discriminates well between MEC = 2 and the others mechanisms, while function 2 discriminates relatively well between MEC = 1 and MEC = 3 pesticides. This confirms the adequacy of the initial MEC-code assigned for pesticides.

Table 3 shows the summary of classification results. The total error (around 7%), which correspond to misclassified compounds between MEC = 1 and 3, was considered satisfactory. Looking at the three highest standardized coefficients, for function 1, $\log k4$ (8.54), $\log k2$ (−6.76) and $E - \log P$ (−2.48), and for function 2, MV (2.53), $\log k4$ (−2.23) and Pol (−1.68), together with the forward variable selection results, it can be concluded that retention factors, at least at two surfactant concentrations, are important in MEC classification for the present set of compounds.

3.3. Fish toxicity–retention relationships

Fig. 3 shows the pLC(r) versus $\log k4$ relationship for all pesticides with available toxicity data in Table 1, labelled according to variable MEC. Fig. 3 also includes four toxicity levels assigned to the pLC_{50} values in fish, taking into account the Directive 3/21/EEC [37]: 'Very toxic pesticides' (pLC_{50} values higher than 0), 'Toxic pesticides' (pLC_{50} values between 0 and −1) and 'Harmful pesticides' (pLC_{50} values between −1 and −2). In the other cases, compounds must be considered 'Safe pesticides'.

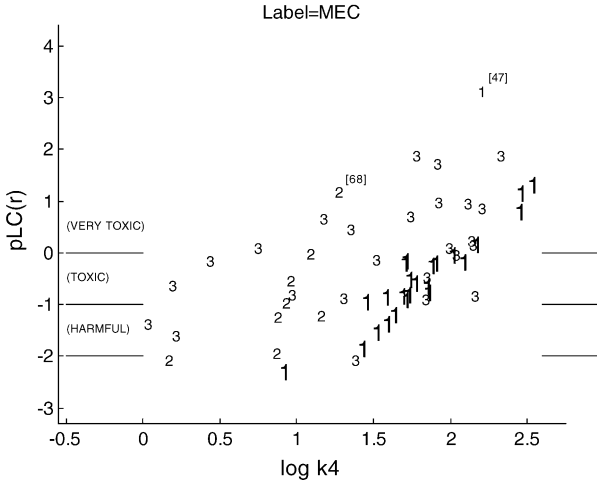

Fig. 3. pLC(r) vs. $\log k4$ relationship for pesticides labelled with the assigned MEC. The toxicity levels have been included. The location of the atypical compounds $N = 47$ and 68 is indicated.

Looking at the global trend, we can conclude that the less retained pesticides ($\log k4 < 1$) are below the very toxic category, while for pesticides with $\log k4 > 1$ toxicity increases with $\log k4$. For MEC = 1 pesticides ($\log k4$ values between 1 and 2.5) a good linear relationship exists. This relationship marks a baseline toxicity, which is an expected result for these non-polar narcotics pesticides, since $\log k$ accounts for hydrophobicity. As shown Fig. 3, Endosulfan ($N = 47$; initially set as MEC = 1) shows a pLC_{50} value too high respect to that baseline toxicity. Some studies reveal that endosulfan is able to inhibit the fish AChE enzyme [38], then its pLC_{50} value agrees with a MEC = 3, in contrast to the rest of FAM = 4. This agrees with the fact that the cyclodiene structure of endosulfan is very different that the structure for the rest of FAM = 4 pesticides (aromatic chlorines).

There is not a defined pLC(r) versus $\log k4$ relationship for MEC = 2 and MEC = 3 pesticides. Some of them show pLC_{50} values close to the baseline toxicity, while others show larger values (up to 3-pLC_{50} units). This behaviour agrees with the reported results in literature, particularly for compounds with reactive toxicity [7], from which pLC_{50} values between 1 and 4 times larger than the baseline toxicity are expected. These results confirm that the previous identification of the toxicity mechanism of action of pesticides is important in order to estimate their toxicity.

3.4. Baseline toxicity—comparative study

The results of Section 3.3 suggests that a single retention measurement (i.e. $\log k4$) could permit to estimate pLC_{50} values in fish for new pesticides, once their mechanism have been assigned/classified as MEC = 1, by means of statistically consistent QRAR models (see below). For MEC ≠ 1 pesticides, the estimations have to be regarded as the minimum toxicity expectable in fish (non-specific toxicity component). In other words, it is possible to define a new baseline toxicity, in terms of $\log k4$ instead to $\log P$.

Fig. 4 shows a comparison between the 'scatter plots' pLC(r) versus $\log k4$ and pLC(r) versus $E - \log P$ for MEC = 1 compounds. As can be observed, the relationship using $\log k4$ shows higher slope (sensitivity) and lower dispersion than the corresponding $E - \log P$ one. The equations obtained in both cases with the available pLC(r) data (excluding compound $N = 47$; see Section 3.3) were:

$$\mathrm{pLC}(r) = -4.5(\pm 0.6) + 2.3(\pm 0.4)\ \log k4, \qquad (2)$$

$$n = 24,\ r^2 = 0.89,\ F = 172.7,\ p < 0.0001,$$

$$\mathrm{RMSEC} = 0.28,\ \mathrm{RMSECV} = 0.33,$$

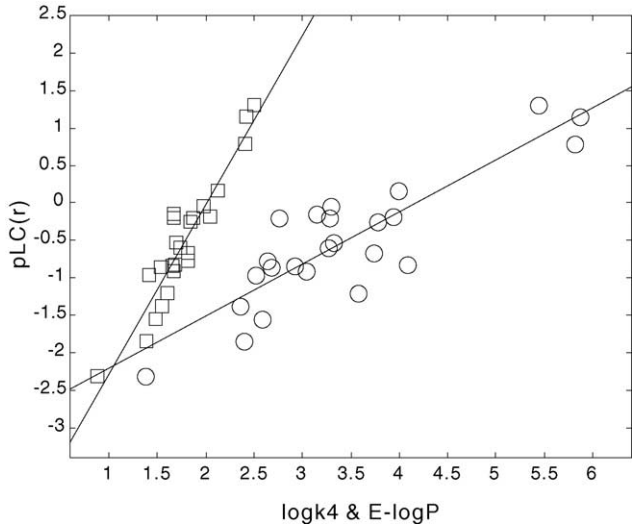

Fig. 4. pLC(r) vs. log $k4$ (□) and $E - \log P$ (◯) relationships for MEC = 1 compounds excluding the compound $N = 47$. Lines represent the linear regression fits.

and

$$\mathrm{pLC}(r) = -2.9(\pm 0.6) + 0.69(\pm 0.17)E - \log P, \quad (3)$$

$$n = 24,\ r^2 = 0.76,\ F = 71.5,\ p < 0.0001,$$

$$\mathrm{RMSEC} = 0.41,\ \mathrm{RMSECV} = 0.42.$$

This indicates that log $k4$ fits better the pLC(r) data (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 it has higher predictive ability (lower root-mean-square error in cross-validation, RMSECV) than $E - \log P$. These results could reflect the log P contribution to the uncertainty of the QSAR model (Section 3.1). Similar conclusions were found when toxicity was expressed as $\log \mathrm{LC}_{50}$, in mmol L^{-1} (original baseline toxicity proposal [6]), and when pLC(b) instead of pLC(r) data were used. The models using log $k2$ or log $k6$ were equivalent to that using log $k4$. Initially, extrapolation of k at null Brij35 concentration in the mobile phase (i.e. log $k0$) would be an alternative to the use of log $k4$ (or log $k2$ or log $k6$) for the estimation of pLC_{50} values. The only critic aspect to do this is that extrapolation could introduce extra uncertainty into the model, so this aspect deserves more attention.

3.5. Comparison of approaches to estimate fish acute toxicity

In order to estimate the acute toxicity in fish for new pesticides several approaches can be used. Unfortunately, in vivo acute toxicity test is very expensive, difficult (which contribute to the inter-laboratory uncertainty and can explain the variability of data in Fig. 1) and finally time-consuming. Fast toxicity estimations can be obtained using software (i.e ECOSAR) that provides pLC_{50} values introducing some molecular data of the new pesticides. A caution with the use of ECOSAR is that some compounds can have more than one chemical class (ECL-value); so different pLC_{50} estimations could be obtained (in some cases they can differ in more than one pLC_{50} units).

Table 1 provides the ECOSAR estimates for all pesticides ($E - \mathrm{pLC}$), which can be compared with the experimental ones, i.e pLC(r). A plot of pLC(r) versus $E - \mathrm{pLC}$ (not shown) reveal that reasonable agreement is only observed for MEC = 1 compounds. This is a logic result, since ECOSAR is a QSAR approach based on log P. For MEC = 2 and most of the MEC = 3 pesticides, ECOSAR provides pLC_{50} estimations lower than the experimental values. This indicates that ECOSAR tends to 'label' the pesticides as less toxic than they are respect to the experimental data from ECOTOX. For instance, using the ECOSAR approach, dimethoate ($N = 4$; $E - \mathrm{pLC} = -2.146\ \mathrm{mg\ L^{-1}}$), would be classified as a 'safe'-pesticide, while according the ECOTOX data, $\mathrm{pLC}(r) = -0.834\ \mathrm{mg\ L^{-1}}$, it would be considered a 'toxic'-pesticide.

In addition, for ionic compounds (FAM = 5 and MEC = 2), ECOSAR estimates the pLC_{50} values systematically as one unit lower than the toxicity baseline in order to take into account the ionisation of these pesticides (internal ECL = 4 class criterion). However, this approach leads to estimated toxicity values lower than the experimental pLC_{50} ones (Table 1). All these results suggest that using ECOSAR information could lead to wrong conclusions in most of the cases.

Relatively simple approaches are those based on QSAR (i.e. Eq. (3)) or, as we propose in this work, QRAR models (i.e. Eq. (2)), using one descriptor. They provide an estimation of the non-specific toxicity and are adequate for MEC = 1 pesticides. Table 4 shows the pLC(r) estimations for non-polar narcotic pesticides with no available experimental data from ECOTOX. The table also includes the $E - \mathrm{pLC}$ estimates from ECOSAR for these compounds for comparative purposes. The pesticides have been ordered according their estimated toxicity (pLC_{50} from log $k4$; Eq. (2)) and classified in different toxicity levels consistent with Directive 93/21/EEC [37]. In agreement with results in Section 3.4, estimations based on log $k4$ are more precise than those from log P. We are convinced that the use of approach based on Eq. (1) to obtain log k contributes to this situation. ECOSAR estimations do not include uncertainty, which is a very inconvenient situation in terms of quality policy.

As can be seen different toxicity levels are found depending on the approach selected. For instance, compounds $N = 79$ and 33 (classified as 'harmful' according to their log $k4$ value and ECOSAR) could be classified as 'Toxic' according to their log P. Compounds $N = 49$ and 52 would be classified as 'Very toxic' according to log $k4$; in contrast, according to their log P and the uncertainty limits involved in its toxicity estimates, there would be doubts between the 'Toxic and 'Very toxic' categories. The results of Table 4 point out that

Table 4
pLC_{50} (mg L^{-1}) estimated by log k (BMC-QRAR model; Eq. (2)) and log P (QSAR model; Eq. (3)), with uncertainties in terms of Confidence Intervals (NC = 95%) related to the linear regression model, and ECOSAR ($E - pLC$; Table 2)

N	pLC_{50} estimated from log $k4$	pLC_{50} estimated from log P	pLC_{50} estimated from ECOSAR
Harmful			
73	−1.8 ± 0.2	−1.4 ± 0.3	−2.13
72	−1.44 ± 0.19	−1.5 ± 0.3	−2.14
77	−1.17 ± 0.16	−1.1 ± 0.2	−1.60
79	−1.08 ± 0.15	−0.9 ± 0.2	−1.40
85	−1.08 ± 0.15	−2.3 ± 0.5	−2.39
74	−1.07 ± 0.15	−1.3 ± 0.3	−1.96
33	−1.00 ± 0.14	−0.9 ± 0.2	−1.43
Toxic			
84	−0.52 ± 0.13	−1.1 ± 0.2	−1.62
35	−0.49 ± 0.13	−0.63 ± 0.18	−1.03
41	−0.15 ± 0.14	0.0 ± 0.2	−0.19
50	−0.14 ± 0.14	−0.2 ± 0.2	−0.31
46	−0.13 ± 0.14	−0.61 ± 0.18	−0.83
83	−0.12 ± 0.14	0.0 ± 0.2	−0.29
Very toxic			
49	0.27 ± 0.18	−0.2 ± 0.2	−0.31
53	0.5 ± 0.2	0.7 ± 0.4	0.76
52	0.5 ± 0.2	0.2 ± 0.2	−0.42
54	0.8 ± 0.2	1.2 ± 0.5	1.30

the approaches are not equivalent, thus affecting the decision-making step in toxicity studies.

For pesticides with other mechanisms of action different from MEC = 1, new QSAR models that incorporate other molecular descriptors could be investigated. Such models will incorporate the uncertainty of the new descriptors making the model less reliable, so the use of log $k4$, that introduces low and controlled uncertainty, should be preferable. Such approaches combining chromatographic and structural information would compete with approaches like ECOSAR that has shown to be not adequate to estimate pLC_{50} of most pesticides.

4. Conclusions

The use of the retention factor in biopartitioning micellar chromatography to estimate the toxicity level of pesticides in fish is evaluated. The results for three families of pesticides, whose mechanism of action in fish is non-polar narcosis, are consistent and permit to develop a simple QRAR model to perform precise pLC_{50} estimations of new pesticides. For pesticides with others mechanisms of action, only qualitative observations (RAR) could be derived with the present approach. In addition, retention data at other surfactant concentrations are useful in the classification of mechanism of action of pesticides in fish.

The developed QRAR model, which uses a unique chromatographic descriptor, log $k4$, has superior quality than that a parallel QSAR model using log P, therefore, it permit to extrapolate usefulness of log $k4$ in other more complex QSAR models for general proposes. The actual approach can be considered as an alternative in vitro method to estimate the non-specific fish toxicity level for pesticides. These results permit to encourage future investigations using different organisms (daphnia, algae) or different toxicological endpoints (soil adsorption coefficient, bioconcentration factor), in order to complete a hazard assessment strategy. Chromatographic retention in BMC seems to be a practical tool to perform such studies, mainly due to its simplicity, low cost and adequate precision (work to establish such relationships is in progress).

Acknowledgements

The authors acknowledge the Spanish Ministry of Science and Technology (MCYT) and the European Regional Development Fund (ERDF) for the financial support (Project SAF2002-01330). The authors also acknowledge the Generalitat Valenciana (Grupos 04/02) for the financial support. J.M. Bermúdez Saldaña is grateful to the Ministry of Education, Culture and Sports for the FPU Grant (AP2001-3088).

References

[1] P. Gramatica, A. Di Guardo, Chemosphere 47 (2002) 947.
[2] Commission of the European Communities 1992, Official Journal L 383 A, EEC Directive 92/69/EEC.
[3] M. Repetto, Toxicología Avanzada, Díaz de Santos, Madrid, 1995.
[4] T.W. Schultz, M.T.D. Cronin, J.D. Walker, A.O. Aptula, Teochem 622 (2003) 1.
[5] H. Könemann, Toxicology 19 (1981) 209.
[6] G.D. Veith, D.J. Call, L.T. Brooke, J. Can, Fish Aquat. Sci. 40 (1983) 743.
[7] H.J.M. Verhaar, J. Solbé, J. Speksnijder, C.J. Van Leeuwen, J.L.M. Hermens, Chemosphere 40 (2000) 875.
[8] W.M. Meylan, P.H. Howard, User's Guide for the ECOSAR Class Program. http://www.epa.gov/oppt/newchems/21ecosar.htm.

[9] C. Hansch, Comprehensive Medicinal Chemistry, vol. 6, first ed., Pergamon Press, New York, 1990.
[10] K. Vandecasteele, I. Gaus, W. Debreuck, K. Walraevens, Anal. Chem. 72 (2000) 3093.
[11] J.G. Dorsey, M.G. Khaledi, J. Chromatogr. A 656 (1993) 485.
[12] M.St.J. Warne, D.W. Connell, D.W. Hawker, Chemosphere 19 (1989) 1113.
[13] J. Dai, J. Lijun, S. Yao, L. Wang, Chemosphere 42 (2001) 899.
[14] M.G. Khaledi, E.D. Breyer, Anal.Chem. 61 (1989) 1040.
[15] A. Taillardat-Bertschinger, P.A. Carrupt, F. Barbato, B. Testa, J. Med. Chem. 46 (2003) 655.
[16] C. Quiñones Torrelo, Y. Martín Biosca, J.J. Martínez Pla, S. Sagrado, R.M. Villanueva Camañas, M.J. Medina Hernández, Mini Rev. Med. Chem. 2 (2002) 145.
[17] L. Escuder Gilabert, J.J. Martínez Pla, S. Sagrado, R.M. Villanueva Camañas, M.J. Medina Hernández, J. Chromatogr. B 797 (2003) 21.
[18] Patent in Process, Number P200002045, Registered to the Universitat de València.
[19] Y. Martín Biosca, L. Escuder Gilabert, M.L. Marina, S. Sagrado, R.M. Villanueva Camañas, M.J. Medina Hernández, Anal. Chim. Acta 443 (2001) 191.
[20] L. Escuder Gilabert, Y. Martín Biosca, S. Sagrado, R.M. Villanueva Camañas, M.J. Medina Hernández, Anal. Chim. Acta 448 (2001) 173.
[21] ACD Labs™ Software (version 5.12), Advanced Chemistry Development Inc., Toronto, Canada, http://www.acdlabs.com.
[22] L. Escuder Gilabert, J.M. Bermúdez Saldaña, R.M. Villanueva Camañas, M.J. Medina Hernández, S. Sagrado, J. Chromatogr. A 1033 (2004) 247.
[23] J.A. García Domínguez, J.C. Díez Masa, Pure Appl. Chem. 73 (6) (2001) 969.
[24] ECOTOX database, US EPA, Mid-Continent Ecology Division, Duluth, MN (MED-Duluth), http://www.epa.gov/ecotox.
[25] PhysProp Database, Syracuse Research Corporation, http://esc.syrres.com/intekow/PhysProp.htm.
[26] M.T.D. Cronin, T.W. Schultz, Teochem. 622 (2003) 39.
[27] R.S. Boethling, D. Mackay, Handbook of Property Estimation for Chemicals: Environmental and Health Sciences, CRC Press LLC, Boca raton, Florida, 2000.
[28] T.W. Schultz, M.T.D. Cronin, Environ. Toxicol. Chem. 22 (2003) 599.
[29] S.G. Abernethy, D. Machay, L. McCarty, Environ. Toxicol. Chem. 7 (1988) 469.
[30] M.T.D. Cronin, T.W. Schultz, Chemosphere 32 (1996) 1453.
[31] T.W. Schultz, D.T. Lin, T.S. Wilke, L.M. Arnold, in: W. Karcher, J. Devillers (Eds.), Practical Applications of Quantitative Structure—Activity Relationships (QSAR) in Environmental Chemistry and Toxicology, Kluwer Academic, Dordrecht, Netherlands, 1990.
[32] M.T.D. Cronin, J.C. Dearden, Quant. Struct. Act. Relat. 14 (1995) 1.
[33] S. Ren, Chemosphere 53 (2003) 1053.
[34] L. Zychlinski, S. Zolnierowicz, Toxicol. Lett. 52 (1990) 25.
[35] T.W. Schultz, Ecotox. Environ. Saf. 14 (1987) 178.
[36] M. Cajina Quezada, T.W. Schultz, Aquat. Toxicol. 17 (1990) 239.
[37] Commission of the European Communities 1993, Official Journal L 110, EEC Directive 93/21/EEC.
[38] H.M. Dutta, D.A. Arends, Environ. Res. 91 (2003) 157.