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Bioconcentration of Pirimiphos-methyl in Killifish (*Oryzias latipes*)

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Abstract: Killifish (*Oryzias latipes*) were exposed to an organophosphate pesticide, pirimiphos-methyl, in a flow-through system to determine the bioconcentration factor (BCF) following GLP (Good Laboratory Practice). This study was conducted at two different concentrations (1 and 10 μg/L) of ^{14}C-labeled pirimiphos-methyl for 28 days uptake and 14 days depuration according to the OECD 305 test guideline. The BCF_{ss} for total radioactive residues in whole fish were 1,251 and 1,277 for low and high concentrations, respectively. The BCF_k based on the uptake and depuration rate constants were 1,200 for both low and high concentrations. During the depuration phase, the accumulated test substance was rapidly depurated from fish. Greater than 95% of the residue at steady-state was depurated after 2 days. Although the measured BCF values were high, pirimiphos-methyl could be evaluated as a low risk from bioaccumulation by aquatic organisms due to the short depuration period and low amount of bound residue (1.5%). We suggest that in evaluating bioaccumulation, not only the BCF should be considered, but also depuration time and bound residue in aquatic organisms give an indication of the potential environmental risks.

Key Words: Pirimiphos-methyl, Bioconcentration factor (BCF), Uptake and depuration rate constants, Steady-state, Bound residue

INTRODUCTION

The increasing use of pesticides has resulted in more chemicals being introduced and discharged into the environment. Bioconcentration is the process of accumulation of water-borne chemicals by fish and other aquatic animals through non-dietary routes[1)]. The bioconcentration factor (BCF) is formally defined as the ratio of the concentration of chemical present in fish at steady-state and the concentration in water and/or as the ratio between the uptake and depuration rate constants[2)]. The BCF is an important parameter that describes the tendency of chemicals to concentrate in aquatic organisms[1, 3)]. An important purpose of REACH system, Registration, Authorization and Evaluation of Chemicals, is to generate toxicity data for previously untested chemicals[4)]. Therefore, Nordberg and Ruden (2007) investigated potential consequences of using BCF as a tool for priority setting in chemicals control. In general, BCF can be determined by either experimental measurement using flow-through system[5-8)] or estimation using regression equation between BCF and octanol/water partition coefficient (log K_{ow})[3,9-12)]. Because experimental method is expensive and time consuming, most researchers have used the prediction method for determining BCF[10,12)]. However, the experimental determination will lead to a better understanding of bioconcentration process in a real biological environment. Also, REACH has recommended that the assessment of bioaccumulation shall be based on measured data on bioconcentration in aquatic species to identify the bioaccumulation criteria[13)].

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Pirimiphos-methyl is an effective organophosphate insecticide and acaricide against various pests with contact and respiratory action. Pirimiphos-methyl can cause cholinesterase inhibition and has used as a post-harvest insecticide. Although pirimiphos-methyl is toxic to birds (acute oral LD_{50} for bobwhite quail, 40 mg/Kg) and fish (96h LC_{50} for rainbow trout, 0.64 mg/L)[14, 15], these toxicities are not of concern based on the use pattern of limited exposure and consequently the risk is low. However, based on its $\log K_{ow}$ value (4.2 at 20℃), it requires evaluation for its bioaccumulation potential.

Therefore, the objective of this study is to determine the bioaccumulation potential of pirimiphos-methyl by determining the bioconcentration factor (BCF), of a ^{14}C-labeled pirimiphos-methyl based on the distribution of the total residues at steady-state in water and whole fish body and the depuration rate from fish in a study conducted under a good laboratory practice (GLP) system.

MATERIALS AND METHODS

Chemicals

Pirimiphos-methyl (*O*-2-diethylamino-6-methylpyrimidine-4-yl *O*,*O*-dimethyl phosphorothioate, 99.2% of purity) and ^{14}C-labeled pirimiphos-methyl (99.3% of radio-chemical purity) were supplied by Syngenta (Berkshire, UK). All organic solvents used were of high-performance liquid chromatography (HPLC) grade and purchased from Burdick & Jackson (Korea). The tissue solubilizer (Soluene-350) and cocktails for LSC and RI-HPLC were purchased from PerkinElmer (MA, USA).

Test organism

Killifish (*Oryzias latipes*) were used as test organism according to the Guideline for Bioaccumulation Test Method in the Testing Guidelines for Toxicology Studies and in compliance with TCCA-Good laboratory Practice Standards and Test Guidelines[16]. Killifish (*Oryzias latipes*) were obtained from Ecotoxicology Laboratory in Korea Institute of Toxicology (KIT). The stock population of fish was acclimated for seven days under the test conditions. During the experimental period, the average length of fish was 3.13 (±0.05) cm for low concentration and 3.17 (±0.08) cm for high concentration. The average weight of fish for low and high concentration were 0.2291 (±0.0291) and 0.2359 (±0.0345) g, respectively.

Test system

A continuous flow-through exposure system delivered dilution water (dechlorinated tap water) and test substance to a surfactant control tank and two test tanks (low and high concentration). The flow rates of dilution water and stock solutions were 200 mL/min and 20 μL/min, respectively, which exchanged approximately 11.5 times of the test tank volume per day. The flow rates of stock solutions and dilution water were checked 48 and 24 hours before the start of the test and then at least 5 times per week during the test.

Measurement of test conditions

According to the OECD guideline, dissolved oxygen (DO), total organic carbon (TOC), total hardness, pH and temperature were measured in all test tanks. Water temperature, DO, pH and flow rate were recorded at least 5 times per week throughout the test. Total hardness was measured at the first day and the last day of experimental period in the control and one test tank at the higher concentration. The TOC throughout the test was measured at both 48 and 24 hours before the start of the test and at least once a week. The fish were fed a commercially prepared food diet (TetraMin®, Tetra in Melle, Germany) throughout the test. The feeding rate was 1 to 2% of the total biomass daily. Uneaten food and feces were removed by siphon. The photoperiod was 16 hours light and 8 hours dark throughout the test.

Determination of the lipid content of the fish

Lipid content was determined in 5 fish from the control tank on days 0 and 42 and from the high concentration tank on days 28 and 42. The analytical method for lipid determination in fish was based on the Bligh-Dyer method[17]. The weighed samples were homogenized for 2 min using a Polytron homogenizer with 3 mL of MeOH:$CHCl_3$ (2:1, v/v). An additional 1 mL of $CHCl_3$ was added and the mixture was homogenized for 30 sec. Deionized water (1 mL) was added to the mixture, and then it was homogenized again for 30 sec. The mixture was filtered through GF/A (Whatman) filter paper, and the remaining tissue was homogenized for 1 min with another 1 mL of $CHCl_3$. After filtering the mixture again, the combined filtrate was transferred to a graduated cylinder and allowed to separate. Then solvent layer was taken off and evaporated in a pre-weighed vessel leaving the lipid which was then

weighed and the percent lipid content was calculated.

Preparation of the test solutions and dosing system

Based on OECD guideline recommendations on selecting test concentrations, the final test concentrations for high and low concentration were set at nominally 10 and 1 μg/L, respectively.

Stock solutions were prepared by mixing labeled and non-labeled compounds (1:1). For the high concentration stock solution, 5 mg (12.4 MBq) of radiolabeled chemical in 3 mL acetonitrile and 5 mg of non-labeled standard solution in 5 mL acetonitrile were added into 100 mL vol-flask. After evaporation of acetonitrile with N_2 gas, 10 drops (approximately 0.17 g) of surfactant (HCO-40) was added and then the 100 mL vol-flask was filled up with deionised water. The low concentration stock solution was prepared similarly with 0.5 mg, radiolabeled chemical and 0.5 mg of non-labeled material. A solvent control stock solution was prepared by adding 10 drops of HCO-40 only to deionised water. The stock solutions were renewed periodically during the uptake phase.

Each stock solution was injected with flow rate of 20 μL/min into dilution water having a flow rate of 200 mL/min to provide the test concentration. Dosing of all stock solutions was stopped after the uptake phase (28 days). Fish in all test tanks were transferred to the clean water tanks with flowing dilution water only after the uptake phase was completed.

Determination of radiochemical activity and stability

The specific activity of original standard solution dissolved in acetonitrile was checked by RI-HPLC. The activity showed 4,984 dpm/20 μL from the analysis of 10,000-fold diluted standard solution.

Because each 100 mL of stock solution was used for approximately 3 days, duplicate 1 mL samples of the high concentration stock solution was taken at 0, 1, 2, 4, 8, 12, 24, 48, and 72 hours to confirm the stability of stock solution. The 1 mL sample was extracted with 10 mL of dichloromethane and then 2 mL was taken from dichloromethane layer and then evaporated with N_2 gas. The residue was dissolved with 10 mL of acetonitrile and an aliquot (10 μL) was counted by RI-HPLC.

Because all samples were analyzed on the sampling date or within 24 hr after storage at -18℃ and the test substance dissolved in solvent was stable in a refrigerator, storage stability tests of sample and standard solution were not considered necessary.

LSC validation and recovery of test substance

The Liquid Scintillation Counter (LS6000TA, BECKMAN) was calibrated using an unquenched standard periodically during the study. A count time of 5 min was used for all water and solubilized fish samples. Background values of control water and fish samples were determined at each sampling date to subtract from low and high concentration exposed samples.

A recovery test of ^{14}C-labeled pirimiphos-methyl from fish was performed at two different concentration levels. For low and high concentration recovery samples, 5 μL and 10 μL of standard solution (25,000 dpm/mL) were added to four replicate fish, respectively. The treated fish were cut into 3-4 pieces with scissor. Soluene-350 (2 mL) was added to each sample and the sealed vials were incubated at 50±1℃ with shaking (150 rpm) for at least 2 hours until all tissues were solubilized. After cooling to room temperature, 1.2 mL of acetic acid and 12 mL of scintillation cocktail (Hionic-fluor) were added and analyzed by LSC for total radioactivity. Recovery test of ^{14}C-labeled pirimiphos-methyl from water was replaced with the result of water analysis during the equilibration phase.

Determination of ^{14}C-labeled pirimiphos-methyl concentration in water and fish

Water sampling and analysis were performed at least 5 times per week. Three aliquots of 5 mL were taken from the middle of each test tank and placed into 20 mL scintillation vials. Then 12 mL of scintillation cocktail (Insta Gel Plus) was added to each vial, shaken and then measured for total radioactivity.

Four fish from each test tank were taken at 0 (1 hr), 1, 2, 4, 7, 10, 14, 17, 21, 24 and 28 days during the uptake phase and 0 (6 hr), 1, 2, 3, 7 and 14 days during the depuration phase. Individual weight and length were recorded before analysis. The analytical procedure was the same with that of the fish recovery test described above.

To determine the concentration of extractable and bound residues in fish, 10 fish from solvent control and the high concentration tank were taken after 25 days exposure. The fish and 10 mL of acetonitrile were added to 50 mL volume tube and then homogenized

using a grinder (Polytron®, Switzerland). After shaking for 30 min, the sample was filtered through filter paper (GF/A, Whatman®, England). The filtered solution was evaporated under vacuum to dryness. The residue was dissolved with 1 mL of acetonitrile and an aliquot of 10 μL was injected into RI-HPLC to determine the concentration of solvent extractable residue. The debris was placed into 20 mL scintillation vial and then analyzed by the method of fish recovery test to determine the concentration of bound residue in fish.

Calculation of BCF

The BCF, uptake (*ku*) and depuration (*kd*) rate constants were calculated by the following equations;

The steady-state bioconcentration factor (*BCFss*):

$$BCF_{ss} = \frac{C_f \text{ at steady-state}}{C_w \text{ at steady-state}}$$

The kinetic bioconcentration facor (*BCFk*):

$$BCF_{k} = \frac{ku}{kd}$$

Uptake (*ku*) and depuration (*kd*) rate constants by computer method:

$$C_f = C_w \times \frac{ku}{kd} \times (1 - e^{-kdt}) \quad 0 < t < t_c$$

$$C_f = C_w \times \frac{ku}{kd} \times (e^{-kd(t - t_c)} - e^{-kdt}) \quad t > t_c$$

Where t_c = time at the end of the uptake phase
C_f = concentration in fish
C_w = concentration in water

RESULTS AND DISCUSSION

Test conditions

The flow rates of the combined dilution water and stock solution were in the range of 197-204, 198-202, and 197-202 mL/min for solvent control, low and high concentrations, respectively (Table 1). Temperature ranged from 22.7-24.2, 23.2-24.2, and 22.3-24.2℃ (Table 1), dissolved oxygen from 6.3-7.2, 6.2-7.1, and 6.3-7.3 mg/L (Table 1), pH from 6.9-7.2, 6.8-7.2, and 6.8-7.2 (Table 1) for the solvent control, low, and high concentration tanks, respectively. Total hardness as $CaCO_3$ were 46.3 and 46.7 mg/L for solvent control and 46.6 and 46.7 mg/L for high concentration tank, taken at the first day and the last day of experimental period, respectively (Table 1). Throughout the experiment, all TOC values were below the detection limit (0.5 mg/L).

Determination of lipid contents

The lipid contents were determined in 5 fish from the control at day 0 and 42 and from the high concentration

Table 1. Test conditions

Items	Mean±SD		
	Solvent Cont.	Low Conc.	High Conc.
Flow rate (mL/min)	199.7±1.8	199.9±1.3	200.1±1.1
Temperature (℃)	23.6±0.4	23.6±0.3	23.6±0.4
Dissolved oxygen (mg/L)[1)]	6.9±0.2	6.8±0.2	6.9±0.3
pH	7.1±0.1	7.0±0.1	7.0±0.1
Total hardness (mg/L)[2)]	46.3 and 46.7	-	46.6 and 46.7
TOC (mg/L)	< 0.5[3)]	< 0.5	< 0.5

[1)] Saturated value: 8.58 mg/L at 23℃
[2)] Values as $CaCO_3$ were from samples on the first day and the last day of experimental period
[3)] Detection limit

Table 2. Lipid contents

Test tank	Expose time (day)	Lipid contents (%)					Mean±SD
		1	2	3	4	5	
Solvent Cont.	0	8.7	6.7	9.0	5.2	6.3	7.2±1.6
	42	8.1	9.4	4.1	3.6	9.8	7.0±2.9
High Conc.	28	7.3	8.7	7.0	6.1	6.6	7.1±1.0
	42	7.9	3.9	5.5	8.6	5.7	6.3±1.9

at day 28 and 42. The average lipid contents were 7.2 and 7.0% for control fish and 7.1 and 6.3% for exposed fish (Table 2). There were no significant changes of fish lipid content during the experimental period.

Stability of stock solutions

To confirm the stability of stock solution, the samples taken at 0, 1, 2, 4, 8, 12, 24, 48, and 72 hours from the high concentration stock solution were analyzed by RI-HPLC after solvent extraction. The average recovery rate was 108.1±5.6% (99.3-114.8%) (Table 3). This result means that ^{14}C-labeled pirimiphos-methyl was stable in the water based medium for at least 3 days.

Recovery

Acceptable recoveries were obtained from fish and water. The recoveries of ^{14}C-labeled pirimiphos-methyl from fish were 111.6±12.4 and 118.2±7.3% at the two different concentration levels (Table 4). Measured concentrations in water during the equilibration phase were 88.9±1.3 and 99.1±8.5% of nominal for the low concentration and 98.2±2.5 and 90.5±0.7% for high concentration (Table 5). The standard deviation was low enough for each matrix to confirm the precision of analytical procedure at two different concentrations.

Concentration of ^{14}C-labeled pirimiphos-methyl in water and fish

The concentration of ^{14}C-labeled pirimiphos-methyl from test tanks was determined by LSC during the uptake phase. The ranges were 0.45-0.55 and 4.44-5.47 μg/L for low and high concentrations, respectively (Table 5 and Fig. 1 and 2). Those values could be converted to 0.90-1.10 and 8.89-10.71 μg/L based on the ratio of labeled and non-labeled of 1:1. The average concentrations during steady-state, from day 4 to day 28, were 0.50

Table 3. Stability of ^{14}C-pirimiphos-methyl in the high concentration stock solution

Hours	Radioactivity (dpm/10μL)			% Recovery
	1	2	Average	
0	1616	1656	1636	111.3
1	1312	1608	1460	99.3
2	1576	1648	1612	109.7
4	1672	1704	1688	114.8
8	1608	1744	1676	114.0
12	1544	1424	1484	101.0
24	1368	1776	1572	106.9
48	1664	1624	1644	111.8
72	1496	1568	1532	104.2
Mean±SD				108.1±5.6
Coefficient Variation (%)				5.2

Nominal activity: 1470 dpm/10μL

Table 4. Recovery of ^{14}C-pirimiphos-methyl in fish

Level	Nominal (dpm)	Detected (dpm)[1)]	% Recovery	Mean±SD
Low	125	118.86	95.1	111.6±12.4
		147.40	117.9	
		154.59	123.7	
High	250	136.91	109.5	118.2±7.3
		287.49	115.0	
		274.12	109.6	
		305.05	122.0	
		315.35	126.1	

[1)] Values were adjusted by those of control

Table 5. Concentration of ^{14}C-pirimiphos-methyl in water

Phase	Time (days)	Low (mean±SD)		High (mean±SD)	
		dpm/mL[1)]	μg/L[3)]	dpm/mL[1)]	μg/L[3)]
Equilibration	-2	65.30±0.98	0.44±0.01	722.79±18.56	4.91±0.13
	-1	72.95±6.28	0.50±0.04	665.86±5.41	4.52±0.04
Uptake	0	66.68±2.93	0.45±0.02	653.92±5.27	4.44±0.04
	1	68.81±6.79	0.47±0.05	731.09±6.34	4.97±0.04
	2	75.26±4.52	0.51±0.03	776.89±6.32	5.28±0.04
	3	70.29±1.13	0.48±0.01	774.80±3.47	5.26±0.02
	4[2)]	71.14±1.25	0.48±0.01	728.04±8.59	4.95±0.06
	5	67.36±2.04	0.46±0.01	805.10±13.44	5.47±0.09
	7	72.48±2.68	0.49±0.02	778.00±6.72	5.29±0.05
	8	75.76±3.40	0.51±0.02	796.05±10.23	5.41±0.07
	9	73.55±1.81	0.50±0.01	767.42±9.48	5.21±0.06
	10	72.76±0.26	0.49±0.01	768.45±8.84	5.22±0.06
	11	71.65±5.18	0.49±0.04	763.13±4.11	5.19±0.03
	14	70.69±1.45	0.48±0.01	785.41±3.10	5.34±0.02
	15	71.42±3.85	0.49±0.03	772.92±3.07	5.25±0.02
	17	72.24±4.26	0.49±0.03	723.28±4.74	4.91±0.03
	18	76.64±3.37	0.52±0.02	755.56±5.57	5.13±0.04
	21	78.60±5.50	0.53±0.04	774.68±7.18	5.26±0.05
	22	72.30±5.81	0.49±0.04	758.43±0.28	5.15±0.01
	23	69.23±4.09	0.47±0.03	765.69±8.68	5.20±0.06
	24	66.56±0.94	0.45±0.01	764.19±12.46	5.19±0.09
	25	77.63±5.26	0.53±0.04	774.73±5.45	5.26±0.04
	28	81.04±8.08	0.55±0.06	788.51±14.52	5.36±0.10
Average uptake concentration (μg/L)		0.49±0.03 (5.39%)[4)]		5.18±0.22 (4.23%)	
Average steady-state concentration (μg/L)		0.50±0.03 (5.31%)		5.22±0.14 (2.72%)	

[1)] Values were adjusted by those of control samples
[2)] Bold-face means the times of steady-state
[3)] Values were not included the concentration of non-labeled compound
[4)] Numbers in parentheses are the Coefficient Variation (CV).

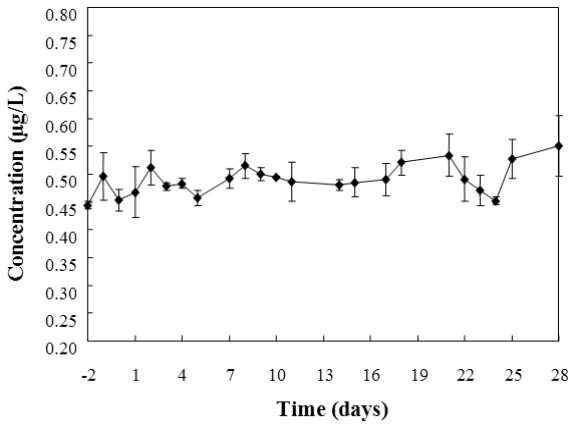

Fig. 1. Changes of ^{14}C-pirimiphos-methyl concentration in water at low concentration during the uptake phase. This figure only showed the concentration of labeled compound in water.

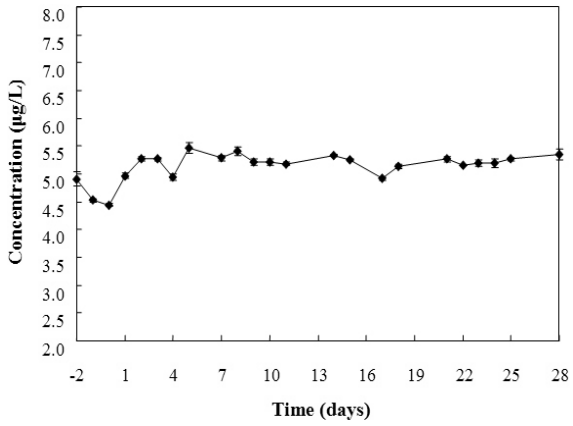

Fig. 2. Changes of ^{14}C-pirimiphos-methyl concentration in water at high concentration during the uptake phase. This figure only showed the concentration of labeled compound in water.

±0.03 and 5.22±0.14 μg/L for low and high concentration, respectively. Detailed data are given in Table 5 which showed just the concentration of labeled compound detected without the concentration of non-labeled compound.

The ^{14}C-labeled pirimiphos-methyl was rapidly accumulated in killifish (Fig. 3). The steady-state was reached after 4 days exposure in both low and high concentration levels. The average concentration at steady-state of the high concentration level was 6666±728 μg/Kg, which was calculated by excluding the outlying value of the 21 day sample to keep the coefficient variation within 20% (Table 6). For the low concentration level, there was more variation at steady-state from 4 days to 28 days. Although the concentration of 21 days sample in low concentration level was excluded, the coefficient variation was 29.2%. However, these results were considered to be acceptable as there were no statistically significant differences between sample dates based on One Way ANOVA analysis using SigmaStat 2.03. There was a statistically significant difference (P = <0.001) between low and high concentration groups.

During the depuration phase, ^{14}C-labeled pirimiphos-methyl was rapidly depurated from fish. More than 50% of the average concentration at steady-state was

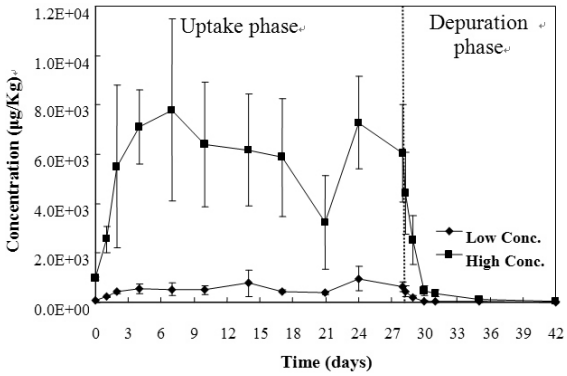

Fig. 3. Changes of ^{14}C-pirimiphos-methyl concentration in fish at low and high concentrations during the study. This figure only showed the concentration of labeled compound in fish.

Table 6. Concentration of ^{14}C-pirimiphos-methyl in fish

Phase	Time (days)	Low (Mean±SD)		High (Mean±SD)	
		dpm/mg[1)]	μg/Kg[3)]	dpm/mg[1)]	μg/Kg[3)]
Uptake	0	12.9±0.7	87.8±4.8	142.4±7.4	967.5±50.4
	1	35.6±7.2	241.6±49.2	375.8±80.2	2,553.1±545.1
	2	61.8±8.0	420.1±54.0	810.2±484.6	5,505.0±3,292.8
	4[2)]	79.5±29.4	540.2±199.6	1,046.4±222.7	7,109.7±1,513.1
	7	78.3±38.0	531.7±258.4	1,147.2±542.5	7,794.4±3,685.8
	10	73.1±26.4	496.7±179.7	939.6±371.0	6,383.9±2,521.0
	14	113.6±77.3	772.2±525.1	909.0±334.7	6,176.0±2,273.8
	17	66.4±10.6	451.4±71.7	864.3±350.5	5,872.2±2,381.6
	21	56.7±9.1	385.6±62.0	477.0±279.9	3,240.7±1,901.6
	24	142.0±73.6	965.1±499.8	1,071.3±276.9	7,279.1±1,881.4
	28	91.5±27.7	621.8±188.0	889.7±290.2	6,045.0±1,971.8
Depuration	28.25	65.3±31.1	443.5±211.4	649.5±244.8	4,412.9±1,663.1
	29	30.9±9.4	210.3±63.7	372.8±145.1	2,533.1±985.5
	30	6.5±2.3	44.3±15.5	70.0±27.9	475.4±189.5
	31	4.4±2.2	30.2±14.9	52.6±20.4	357.4±138.9
	35	4.1±2.4	27.8±16.6	14.7±8.2	99.9±55.9
	42	0.9±0.4	6.4±2.7	3.4±0.5	23.3±3.6
Average steady-state concentration (μg/Kg)		625.6±182.5 (29.2%)[4)]		6,665.8±728.2 (10.9%)	

[1)] Values were adjusted by those of control samples
[2)] Bold-face means the times of steady-state
[3)] Values were not included the concentration of non-labeled compound
[4)] Numbers in parentheses are the coefficient variation (CV).

Table 7. Bioconcentration factors (BCF)

Level	*ku*	*kd*	BCF_k	BCF_{ss}
Low concentration	684.041 (193.481)[1)]	0.570 (0.173)	1,200	1,251
High concentration	1,008.410 (468.577)	0.840 (0.412)	1,200	1,277

[1)] Numbers in parentheses are the approximate standard errors of the parameters

depurated within 1 day in both low and high concentrations. Also, more than 95% of the average concentration at steady-state was depurated after 2 days. The results for rapid uptake and depuration indicated that the test substance had low bioaccumulative potential.

The concentrations of the bound and extractable residues in fish at steady-state (25 days) were 99.4 and 4,919 μg/Kg, respectively. These values were 2 and 98% of the concentration of total radioactive residue, respectively. From the analysis of solvent extractable solution, no significant peak other than the parent was found in RI-HPLC chromatogram. The OECD guideline recommends metabolites representing over 10% of total radiolabeled residues in fish should be identified and quantified[2)] and there were clearly none. A negligible amount of bound residue suggested that the test substance was not altered on the bioaccumulation.

Determination of BCF

The bioconcentration factor at steady-state (BCF_{ss}) was calculated from the ratio of C_{fish}/C_{water}. The BCF_{ss} were 1,251 and 1,277 for low and high concentration level, respectively (Table 7). Uptake and depuration rate constants (*ku* and *kd*) were calculated by using SPSS 14.0, NLIN. Their values were 684 and 0.570 for the low concentration and 1,008 and 0.840 for the high concentration, respectively. Therefore, the ratios of *ku*/*kd* (BCF_k) were 1,200 for both low and high concentrations. REACH Annex XII indicates that a BCF higher than 2,000 fulfils the bioaccumulation criterion, which is one of PBT properties (Persistency, Bioaccumulation and Toxicity)[13)]. Therefore, the above BCF values indicated that the test substance would not be classified as PBT chemical.

CONCLUSION

During the uptake phase, the concentration of ^{14}C-labeled pirimiphos-methyl was rapidly increased in fish, reaching a steady-state within 4 days. The BCF_{ss} for total radioactive residues in whole fish were 1,251 and 1,277 for low and high concentrations, respectively. The BCF_k based on the uptake and depuration rate constants were 1,200 and 1,200 for both low and high concentrations. During the depuration phase, ^{14}C-labeled pirimiphos-methyl was rapidly depurated from fish. Greater than 95% of the average concentration at steady-state was depurated after 2 days. To assess the risk of bioaccumulation, Franke *et al*. (1994) proposed that the classification of bioaccumulation into 4 categories that considers not only the BCF, but also the complexity of bioaccumulation processes including elimination half-life time (CT_{50}), organ specific bioaccumulation, and bound residues[18)].

In conclusion, although the pirimiphos-methyl showed limited bioconcentration potential in killifish (*Oryzias latipes*), the bioconcentrated residues were rapidly eliminated in the clean water. Also, the amount of bound residues was negligible. Therefore, bioaccumulation potential of the pirimiphos-methyl could be considered to be low.

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