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Bioconcentration kinetics of PCBs in various parts of the lifecycle of the tadpoles *Xenopus laevis*

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Abstract

Polychlorinated biphenyls (PCBs) in *Xenopus laevis* have been reported only for a few congeners. Additionally, there is very little information on the ability of *Xenopus laevis* to bioconcentrate PCBs. To address these issues, the tadpole *Xenopus laevis* was exposed to Aroclor1254 mixtures in water at room temperature for 110 d followed by an additional 110 d of nonspiked PCBs in the water for the control group. During the whole process, bioconcentration factors (BCFs) of PCBs ranged from 1180 to 15670. For most PCB congeners, the highest and lowest bioconcentrations of the kinetic curves were found to be remarkably simultaneous, respectively. All 141 PCB congeners under the same experimental conditions had no linear correlation on the lgBCF versus lgK_{ow} relationship. The relationship between lgBCFs and lgK_{ow} followed a parabolic pattern indicative of selective bioconcentration, suggesting that the kinetic curves of the PCB congeners observed in the lifecycle of the tadpoles may be concentrated due to the amphibian special species and internal metabolism. In contrast, lgBCFs for PCBs were inversely related to lgK_{ow}, suggesting that a metabolism of the higher K_{ow}' PCB congeners occurred. These results support the author's conclusion that the tadpole *Xenopus laevis* plays major roles in the bioconcentration of PCB congeners, and demonstrated that the exposure kinetic curves of PCB congeners are complex. Besides the amphibian metamorphous development, the lifecycle of the tadpole *Xenopus laevis* also may be of importance in determining the bioconcentration of PCB congeners.

Key words: PCBs; tadpole *Xenopus laevis*; bioconcentration; exposure kinetics

Introduction

Polychlorinated biphenyls (PCBs) are a class of persistent organic pollutants that exist ubiquitously in the environment. They are resistant to degradation in the environment and are widely distributed in the aquatic ecosystems, moreover, PCBs are highly lipophilic and bioconcentrative in adipose and other lipid-rich tissues of biota (ATSDR, 1998; Cogliano, 1998; Qin *et al*., 2003), although the sale and use of PCBs have been banned in most countries for almost 20 years. They are among the most prevalent environmental pollutants and can be found in various environmental compartments, and still pose a serious threat to aquatic organisms (Connell *et al*., 1998a, b) and continue to cause a lot of ecotoxicological effects.

Recently, considerable attention has been focused on PCBs which can disrupt the functions of the animal and human endocrine systems (Qin *et al*., 2003). These endocrine disruptors may cause a variety of problems regarding growth, development, and reproduction behavior (Kavlock *et al*., 1996). Amphibians might represent potential sentinels for assessing the adverse effects of environmental chemicals because of their permeable skins and biphasic lifecycle (van der Schalie *et al*., 1999). Toxic effects of PCBs as endocrine disruptors on amphibians are becoming the focus of ecological system study. In addition, recent declines in the amphibian population and increases in their developmental abnormalities have stimulated the investigation on these issues (Phillips, 1990; Wake, 1991; Schmidt, 1997). The use of amphibians as sentinel species in toxicity evaluations has been suggested by various investigators (Connell *et al*., 1998a, b; Burkhart and Gardner, 1997). Several authors reported the effects of endocrine disruption on gonadal differentiation in amphibians. Even though tadpole *Xenopus laevis* is a kind of bon model animal widely used in biology and toxicology, there have been little data regarding the changes and regular patterns of PCBs congeners on bioconcentration in the lifecycle of a tadpole. Given the concentrative variety of PCBs in the tadpole in comprising any particular habitat of growth, metamorphous development, the time course of species-specific data available and applicable to exposure pathways, it is important to quantify and characterize the tadpole exposed to PCB at varied stages. The various

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lifecycle stages of the tadpole exposed to PCB mixtures of Aroclor1254 congeners was investigated in this study. To study the bioconcentration of PCB congeners in various lifecycle stages of the tadpoles, the composition of PCBs and the food sources must be clear. This study is to provide information regarding PCBs congeners with the kinetics and the ratio of the bioconcentrations in various parts of the lifecycle of the tadpoles. The article presents the kinetic curves and the concentration distribution of PCB congeners in the tadpoles for 110 d of study. The biouptake, elimination, metabolism, and building up of mathematic approaches of bioconcentration will be discussed on the basis of the kinetic curves of PCBs in tadpoles.

1 Materials and methods

1.1 Breeding and housing

Mature female and male tadpoles were maintained separately in glass tanks containing dechlorinated water at 22±2°C with a 12-h light/12-h dark cycle and fed once a week on chopped pork liver. Breeding was induced by subcutaneous injection of human chorionic gonadotrophin. Males grasped females with forelimbs to perform external fertilization. After eggs were laid, the females and the males were removed from the breeding tank. Fertilized eggs were incubated at 22±2°C with a 12-h light/12-h dark cycle. On the day 5 after fertilization, tadpoles were fed on *Daphnia* twice weekly.

1.2 Exposure to chemicals

On the day 6 following fertilization, healthy tadpoles at NF stage 46/47 among the offspring of a pair of parental frogs were randomly selected for the exposure experiment (Nieuwkoop and Faber, 1956). PCBs Aroclor1254 were dissolved in ethanol to produce stock solutions. The experimental water was prepared by adding the stock solution to dechlorinated water. Previous study shows that tadpoles exposed to a series of PCBs concentrations (5, 10, 20, 40, 80 μg/L) did not exhibit acute toxic response within 10 d. The concentration (10 μg/L), was chosen which was higher than that in the environment, since PCBs are easily absorbed by glass in the environment. The control group received the same amount of ethanol used as solvent. The tadpoles were exposed to PCBs Aroclor1254 mixtures in water at room temperature for 110 d followed by an additional 110 d of nonspiked PCBs in the water for the control group. Both the exposure and control group experienced an additional 110 d of nonspiked PCBs in food.

The other three tanks containing water with spiked PCBs were set as the water control (no frogs) for the exposure group. At the same time, the experimental water was marked as exposure water (EW) and the plain water (PW). Therefore, the bioconcentrative mechanisms and the relationships between the tadpoles and the water could be obtained as the reference of the experimental background value.

Each treatment contained a series of replicated glass tanks with 30 tadpoles per tank containing 18 L of water. All tanks were the same regarding size and shape (30 cm×20 cm×25 cm). The experimental water was changed twice weekly. Replicated experiment was conducted using the offspring of another pair of parental frogs.

1.3 Reagents and materials

All individual PCB congeners are referred to by their IUPAC nomenclatures throughout the manuscript in the study. The mixtures of Aroclor1254, PCB209 were purchased from AccuStandard Inc (New Haven, USA). The five PCB calibration mixtures provide 141 congeners with 10 g/ml of each group congeners in isooctane, obtained from Accustandard Inc (C-CS-XX series in isooctane, USA). All solvents used were purified by distillation of all airtight glassware. Florisil for cleanup of pesticide residues (Dikma Company, USA), 60/100 mesh, was heated 6 h at 600°C in a Muffle furnace and was activated at 130°C for 8 h in the oven and deactivated with 2% distilled water prior to use. The 141 PCB congeners used as calibration standards (external standards) and PCB209 was used as the recovery standard (internal standards).

1.4 Samples collection and cleanup

The tadpoles and the water samples were collected. The water samples and the tadpole samples were used for liquid-liquid extraction or ultrasonic extraction, respectively, followed by cleanup of sulfuric acid silica column separation, and separation of florisil column (Zhao *et al*., 2005).

The water samples were repeatedly extracted thrice with 10 ml dichloromethane. The dichloromethane layers were collected, pooled, and concentrated under the centrifugation, then replaced by petroleum ether and purified by eluting them through concentrative sulfuric acid silica and anhydrous sodium sulfate complex columns. Final elution was performed with petroleum ether and dichloromethane (9:1, v/v) through a florisil column. The eluents were concentrated by K.D. evaporative apparatus. The sample was exchanged into isooctane in a 100 μl vial and sealed for GC analysis.

The tadpole samples were homogenized with equalization of anhydrous sodium sulfate in a mortar and ultrasonically extracted using petroleum ether and acetone (1:1, v/v). The mixed solution was collected after centrifugation, concentrated, and purified similarly to the water samples.

Following homogenization, CB209 were added as internal standards and consisted of 10 ng of each sample. Each sample was calibrated with the external standards of 141 PCB congeners of which each peak was identified by the retention time (Zhao *et al*., 2005).

1.5 Analysis of PCB congeners

The method includes liquid-liquid extraction or ultrasonic extraction, cleanup of sulfuric acid silica column separation, separation of florisil column, and analyses of capillary gas chromatography. Conditions for cleanup of sulfuric acid silica gel column and separation of florisil

column were optimized.

The concentrated purified isooctane solutions were quantified using GC-ECD for the quantitative analysis of PCB congeners. An Aglient6890 gas chromatograph equipped with ^{63}Ni electron-capture detection (μ-ECD) system (Hewlett-Packard, USA) was used for quantitative analysis of the PCB congeners. The analytical quartz capillary chromatographic column was DB-5 (25 m×0.25 mm×0.25 μm i.d. Aglient Company). The gas chromatography conditions were as follows: injection was 1 μl, injector and detector temperature were 280°C and 320°C, respectively. Oven temperature was programmed from initial 60°C, held for 2 min, increased to 150°C at a rate of 15°C/min, when arrived at 150°C, the ascending rate was changed at 2°C/min, reached the column temperature of 280°C. The inlet was operated in splitless mode and nitrogen of high purity was used as the carrier gas, the velocity of carrier gas flow was 1 ml/min. External standard method was applied for quantitative analysis. Internal standard was used for recovery control.

Individual congeners were identified by their retention time in the gas chromatograph (Zhao *et al*., 2005), and concentration of each analyst was computed on the basis of sample volume or weight. Standard reference curves used five-point concentration curves for assurance of the precision and accuracy tests.

1.6 Quality assurance and control

Each biota sample was spiked with PCB209. The mean recoveries were 75%–135% for biotic samples and were 82%–15% for water samples, respectively, and were maintained through the analytical procedure. Detection limits for individual PCB congeners ranged from 0.02–96.9 ng/g for the biota samples fresh weight and were 0.009–15.3 ng/L for water, and the relative standard deviations (RSD) were all below 6.9% (Zhao *et al*., 2005). PCBs concentrations were calculated by summing the masses of individual congeners. The method was successfully used for the analyses of trace PCB congeners in *Xenopus laevis* and water. The results of the quality control showed that the method merits from the reliable investigation.

1.7 Data analysis

The simplest bioconcentration model was only utilized here, it is assumed that uptake and elimination of hydrophobic chemicals follow first-order kinetics (Branson *et al*., 1975; Dick *et al*., 1993), having water and organisms compartments, it is represented by the equation:

$$dC_x(t)/dt = k_1 \times C_w(t) - (k_2+k_m) \times C_x(t) \quad (1)$$

where, t is time (d), $C_x(t)$ is the PCBs concentration in *Xenopus laevis* (ng/g) at time t, $C_w(t)$ is the PCBs concentration in water (μg/L) at time t, k_1 is the uptake rate constant (L/(g·d)), k_2 is the elimination rate constant of the nonbiotransformed chemical (d^{-1}), and k_m is the biotransformation rate constant (d^{-1}). When biotransformation is entirely blocked, k_m will be equal to zero.

In the first period of uptake, when it is assumed that elimination is negligible, the uptake rate constant k_1 can be estimated according to

$$k_1 = (1/C_w) \times (\Delta C_x/\Delta t) \quad (2)$$

The uptake rate constants of the PCB congeners were calculated using Eq.(2), using the average concentrations of the congeners in *Xenopus laevis* and the average concentration in water which C_x and C_w will be discussed as follows.

The BCF is defined as the ratio between the concentrations of the chemical in *Xenopus laevis* and water at steady state, which is equal to the ratio of uptake and elimination rate constant:

$$BCF = C_x/C_w = k_1/k_2 \quad (3)$$

The BCFs of the PCB congeners are determined according to Eq. (3), assuming steady state and using the ratio C_x/C_w , as k_1 and k_2 are not always available.

Usually for fish, a steady-state solution for Equation (1) and (2) was determined by using the average chemical concentration in the water for the data set. For tadpole *Xenopus laevis*, it is unreasonable to establish initial conditions for the calculation of above equation due to the time course of its habitat growing and metamorphous development, to obtain a steady-state solution for Equations (1) and (2) is more complicated and more difficult. Hence, the BCFs in this report are defined and were calculated using the following equations:

$$BCF = C_x/C_w \quad (4)$$

Where $C_x(t)=\frac{1}{\Delta t}\int_t^{t+\Delta t} C_x(t)dt$, C_x is PCB congeners concentration of a segmented kinetic curves in *Xenopus laevis*, divided by the average water $C_w(t) = \frac{1}{(t_2-t_1)}\int_{t_1}^{t_2} C_w(t)dt$, both C_x and C_w are expressed as (measured as ng/g, thereinto C_w unit converting μg/L into ng/g).

The PCBs initial spiked concentration, as the initial water concentration C_w, was used in the calculation of this report for the simplest expression, actually the changes of PCBs concentrations in this case of semi-static experiment are complicated, we gave Fig.1 and Fig.2 which are the changes of the measured concentrations of PCB individual congeners for an in-depth study. It is clear the BCFs will vary as C_w. The initial spiked water concentration was used in the calculation of this report C_w=10 μg/L=10 ng/g, as the initial concentration, therefore the relationship between

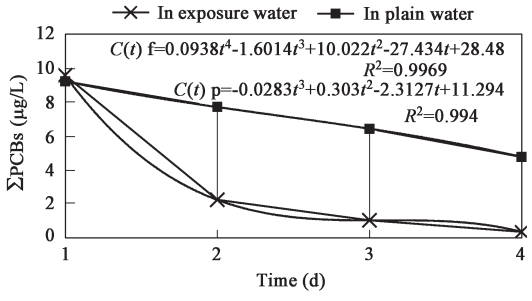

Fig. 1 PCBs measured concentration and curve of analog approach in the water.

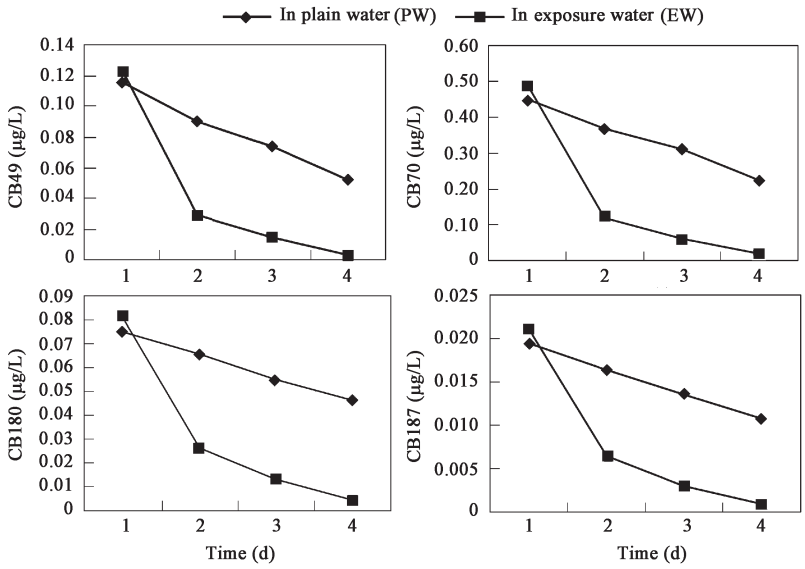

Fig. 2 Concentrations of partial PCB congeners in water.

BCF and C_x is as follows:

$$\mathrm{BCF} = 0.1C_x \quad (5)$$

Bioconcentration factors in this study will be simplified. All the practical measurement concentrations of C_x were used for all the statistics and calculations in the above-mentioned equations. For convenience of study and observation, $C_x(t)$ function is always given to follow up in way of chart or equation, t (d).

In the present study, the actual concentrations of PCB congeners freely dissolved in the water (C_w) are diversified. Regarding PCB congeners, there are no steady states or balanced states in the water (Figs.1 and 2) and thus the measured value C_w is always less than or equal to the actual concentrations of PCB congeners in water. In fact, Eq. (4) used C_w as the initial spiked concentrations more than both the initial and final measured PCBs concentrations in the water, therefore the result would be an underestimation of the BCF approximately because the variety of actual concentration of PCB congeners in water is less than the initial spiked water concentration. No matter what value C_w is considered, the study of *Xenopus laevis* on the law of natural changes and bioconcentrative characteristics will not be affected.

2 Results

The changes of PCBs concentrations in the water are shown in Fig.1, and the concentrations of partial PCB congeners are shown in Fig.2, To sum up, actual initial PCBs concentrations measured in the EW and PW are all less than 10 μg/L of initial spiked concentration, the final concentration of PCBs in the EW was 0.46 μg/L, and concentration of PCBs in the PW reached the final concentration of 4.05 μg/L which used ethanol as solvent for PCBs, respectively (Fig.1).

The results of the changes of partial PCB congeners in the PW in contrast to PCBs concentrations in the EW, at the same time the changes of PCB congeners between the PW and the EW are shown in Fig.2, respectively. For further research on mathematical approach, the changes of PCBs concentrations in water are used for basic reference data only, several mathematical analog approaches are mentioned in the figures to highlight the relationship between the bioconcentrative kinetics and the experiment perfectly as well as reveal a real time course of PCBs in water.

The results of the exposure to chemicals in the water were first given as the bioconcentrative references which involved the future research in the relationship between aquatic circumstances and bioconcentration (another article in detail).

The control groups have not been PCBs components both in biota samples and water samples by measurement. Just like other model animals, the feeding quality control of *Xenopus laevis* is very important with the experimental results scientifically, however, the quality control of *Xenopus laevis* is not very easy to do. To feeding foodstuff *Daphnia*, it must be strictly determinated by GC test every batch foodstuff through the same procedure of chemical analysis with the biota sample, our feeding foodstuff is not contain any PCBs components.

The PCBs levels in *Xenopus laevis* are shown in Table 1. To investigate the differences in chemical concentration in *Xenopus laevis* PCB congeners, the PCBs concentrative curve are shown in Fig.3, one curve of the summation of PCB congeners. Significant differences were found in 110 d for PCBs congeners' data and *Xenopus laevis* contained the highest PCBs body burden on day 110.

The concentrations of the column charts PCB congeners from capillary gas chromatography revealed the presence of 96 individual peaks representing about 141 chlorobiphenyl (CB) congeners on the day 24. Fig. 4 shows that the relative percentage concentrations of CB90+101, CB77+110+154, CB118, CB105, CB138+163+164 are marked beyond the scope of 5% on the day 24. CB52+73, CB70, CB74, CB66+93+95, CB60+91, CB97, CB99, CB81+87+115+117, CB84, CB85, CB134+149, CB153, CB141+179, CB158,

Table 1 PCBs and partial PCB congeners concentrations in *Xenopus laevis* (ng/g, fresh wt) during 110 d

Name	lgK_{ow}	Dec	2 d (n=15)	4 d (n=15)	6 d (n=15)	8 d (n=12)	10 d (n=12)	12 d (n=6)	16 d (n=6)
CB15+18	5.23/5.33	5.26	28.5	1134.9	11.8	410.6	27.5	5.4	≈0
CB28+31	5.71/5.68	8.28	45.0	26.4	43.7	21.6	49.5	30.4	28.7
CB52+73	6.1	2.37	562.3	170.4	314.3	142.9	329.8	209.9	213.5
CB49	6.1	1.38	307.6	101.9	195.2	88.0	203.7	131.2	133.0
CB41+64+71	6.1	1.05	285.1	98.45	169.8	77.4	165.3	111.0	100.7
CB70	5.9	0.44	1586.3	624.8	1174.3	570.9	1179.0	838.4	868.2
CB66+93+95	5.8	0.21	1580.6	647.7	1205.9	571.6	1124.8	831.6	860.2
CB60+91	5.9	0.47	556.4	240.3	449.6	208.3	407.3	300.9	290.6
CB90+101	6.4	2.01	2113.9	1142.0	2005.7	1062.2	1933.3	1479.8	1687.3
CB85	6.18	0.51	740.5	379.7	698.2	355.2	652.4	506.7	519.7
CB99	6.6	0.33	1106.1	613.2	1093.3	586.8	1064.7	824.3	961.0
CB97	6.6	0.53	871.8	391.5	762.5	367.1	708.9	526.9	413.0
CB87+115+81+117	6.5	0.73	1643.1	829.4	1510.8	770.0	1416.4	1088.3	1065.4
CB77+110+154	6.5	0.03	3916.1	1807.0	3383.5	1604.0	2952.7	2266.8	1681.1
CB118	6.71	0.20	4124.9	2319.3	4100.0	2549.4	4517.5	3225.6	4289.9
CB128	6.61	0.10	828.4	576.8	1043.6	590.6	960.6	910.5	817.3
CB151	6.9	0.37	296.2	86.9	138.8	83.5	122.0	109.2	148.1
CB134+149	6.8	1.05	727.3	666.8	1155.7	363.7	483.9	825.1	285.9
CB146	6.8	0.11	166.2	119.7	209.3	123.8	199.5	163.7	193.9
CB153	6.9	0.26	1390.6	1026.0	1783.8	1068.2	1757.9	1442.3	1786.3
CB105	6.4	0.02	2868.8	1532.0	2806.9	1505.4	2572.8	2083.2	2046.0
CB138+163+164	7	0.16	2840.4	1991.0	3497.9	2051.4	3269.3	2774.1	3045.0
CB156	7.4	0.25	651.1	498.5	880.9	536.4	856.3	707.4	803.8
CB158	7.3	0.03	412.7	282.1	516.8	299.2	495.8	413.4	474.0
CB129+178	7.3	0.06	203.2	140.8	255.4	141.1	226.2	190.4	206.2
CB170+190	7.44/7.08	0.05	296.4	292.7	504.9	320.0	474.9	408.5	474.0
CB183	7	0.12	86.9	80.8	132.7	84.9	125.3	107.5	131.2
CB185	7	0.04	5.8	4.8	8.3	5.0	7.7	6.2	7.1
CB157+201	7.44/7.3	0.50	132.2	103.0	173.1	100.9	154.7	130.6	149.1
CB180	7.4	0.05	270.0	264.7	425.9	282.0	421.0	372.4	446.2
CB196+203	7.35/7.49	0.10	21.6	26.7	44.6	30.3	41.7	37.0	42.7
CB194	7.1	0.02	18.1	23.7	39.4	27.0	37.6	32.7	38.2
CB206	7.2	0.16	3.0	3.9	6.5	4.4	5.8	5.4	6.1
PCBs (ng/g)			34939	27732	34966	22270	32915	26044	22598

Name	20 d (n=3)	24 d (n=3)	34 d (n=3)	44 d (n=3)	54 d (n=3)	65 d (n=3)	80 d (n=3)	95 d (n=3)	110 d (n=3)
CB15+18	31.1	28.7	42.4	23.0	20.0	14.6	17.7	21.5	19.1
CB28+31	57.7	51.8	84.9	37.4	60.0	37.8	38.1	102.6	99.0
CB52+73	458.1	496.0	849.3	518.6	613.9	212.8	605.4	952.3	1422.4
CB49	273.3	287.0	492.6	300.1	358.6	119.9	345.5	556.4	909.5
CB41+64+71	218.7	237.2	397.5	253.6	295.0	110.0	295.0	470.9	742.9
CB70	1564.7	1663.3	2525.0	1572.2	1916.8	565.5	1942.1	3276.0	6257.6
CB66+93+95	1569.8	1714.2	2570.4	1568.6	1895.8	572.4	1905.2	3043.8	5756.6
CB60+91	512.4	556.7	844.0	511.7	588.9	194.5	608.8	1028.7	1904.6
CB90+101	2544.9	2738.6	4050.4	2427.3	2966.6	737.5	2705.1	4144.7	11216.5
CB85	796.7	803.8	1250.7	769.5	944.3	263.6	904.0	1587.6	3045.0
CB99	1376.2	1475.9	2214.9	1292.7	1584.3	378.4	1440.3	2409.2	6010.7
CB97	739.7	722.8	966.8	674.1	729.8	299.2	982.7	1675.0	2939.7
CB87+115+81+117	1720.4	1799.1	2549.9	1616.7	1882.0	561.0	2013.6	3111.8	7097.6
CB77+110+154	3264.0	3182.6	4570.4	3241.1	3454.7	1357.5	3880.6	5322.0	11477.1
CB118	5905.0	6200.9	10015.9	5987.9	5602.6	1262.8	4484.2	6074.7	20581.6
CB128	910.5	1171.9	1241.5	1813.0	1201.7	256.0	1044.6	2012.4	4880.7
CB151	169.2	195.0	274.0	162.3	187.2	40.1	160.1	266.9	624.5
CB134+149	491.6	513.1	≈0	≈0	1404.2	365.4	1420.4	563.8	3834.8
CB146	232.3	241.3	369.2	212.4	244.3	53.6	210.8	393.5	1017.7
CB153	2190.3	2190.6	3422.3	1952.1	2264.9	454.4	1181.3	1806.3	6227.5
CB105	3161.6	3467.9	4516.7	2814.4	3333.1	899.6	3053.8	6389.5	13080.6
CB138+163+164	3929.6	4096.1	5909.6	3575.7	4067.3	915.5	3508.6	3440.5	11018.1
CB156	920.1	948.1	1397.6	821.2	931.8	195.7	820.6	1567.8	3951.9
CB158	570.2	562.3	841.1	484.3	550.9	123.1	476.2	2535.7	4941.3
CB129+178	255.7	293.5	427.9	255.8	275.2	68.0	245.3	409.0	869.0
CB170+190	515.6	506.5	808.3	460.9	483.6	86.9	404.2	759.4	1959.8
CB183	133.3	150.8	246.6	137.1	155.4	28.2	127.6	235.4	633.5
CB185	10.7	14.9	23.8	13.6	14.8	3.1	12.3	20.9	50.2
CB157+201	195.7	219.3	312.3	181.3	195.5	30.8	172.8	321.8	798.1
CB180	446.0	439.3	710.7	406.9	440.1	79.0	366.7	690.3	1805.9
CB196+203	41.2	39.1	65.9	38.0	38.8	6.6	31.2	55.7	148.1
CB194	37.1	33.3	58.2	33.5	34.8	5.3	26.3	47.8	128.1
CB206	5.4	4.8	7.8	4.4	4.8	0.7	4.1	8.0	21.9
PCBs (ng/g)	40361	42085	62299	37999	43819	11839	41309	63744	156707

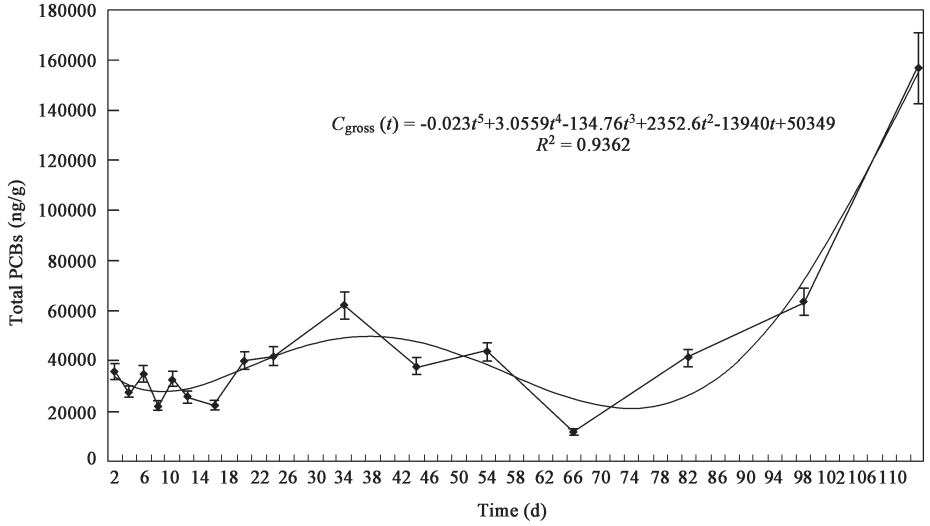

Fig. 3 PCBs measured concentration and curve of analog approach for tadpole *Xenopus laevis*.

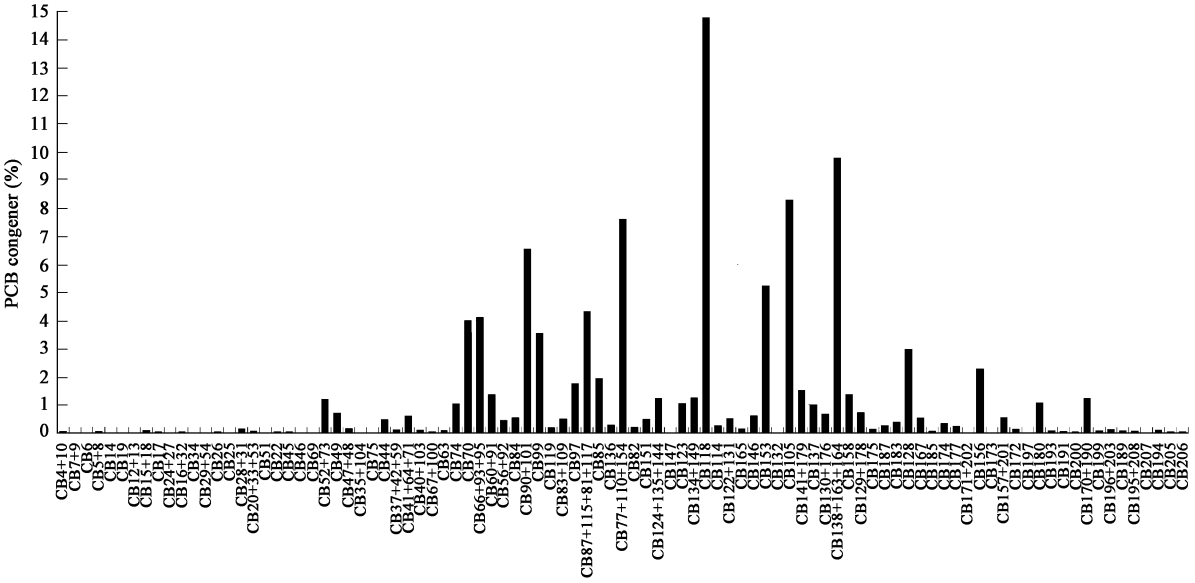

Fig. 4 Relative concentration of PCBs on the day 24.

CB128, CB156 are between 1% and 5%, and the rest CB congeners CB4+10, CB7+9, CB5+8, CB15+18, CB17, CB16+32, CB25, CB26, CB28+31, CB20+33+53, CB22, CB45, CB49, CB51, CB47+48, CB44, CB37+42+59, CB41+64+71, CB40+103, CB67+100, CB59, CB63, CB84, CB56+92, CB119, CB83+109, CB136, CB82, CB151, CB124+135+144, CB123, CB114, CB112+131, CB146, CB137, CB130+176, CB129+178, CB175, CB187, CB183, CB167, CB185, CB174, CB177, CB157+201, CB172, CB180, CB191, CB193, CB170+190, CB199, CB200, CB196+203, CB189, CB195+208, CB194, CB206, CB207 are within the scope of 1% or much less. So are the experimental days similarly etc., the differences in 110 day's concentrative distribution could be due to the amphibious differences in the metamorphous developmental stages of fishes, birds, and mammals. The concentrations of the sum of 141 congeners (PCBs), certain mono-ortho and nonortho-substituted PCBs and the individual congener concentration in the samples on a fresh weight are shown in Fig.4. Throughout the values of practical measurement, in the water (EW) the changes of initial and end PCBs concentrations ranged from 9.59 μg/L to 0.46 μg/L, and the lowest and highest concentrations in *Xenopus laevis* ranged from 11.84 μg/g of day 65 to 156.71 μg/g of day 110. At the highest concentrative level, CB105 and CB118 in *Xenopus laevis*, the concentrations reached 13.08 μg/g to 20.58 μg/g on the fresh weight basis (arithmetic mean value), respectively.

A marked tendency of the concentration of PCBs in all 110 d was obtained, as shown in Fig.3. A significant increase of PCBs from day 65 to day 110 was observed. The PCBs concentrations was not enhanced between 6 and 10 d, 55 and 65 d, and the concentrations in *Xenopus laevis* were not equal or unbalanced to individual congeners concentration during the different developmental stages.

From Table 1 and Fig.4, the medium chlorine-substituted PCB congeners, such as CB66 +93+95, CB70, CB85, CB99, CB97, CB118, CB105, CB128, CB153, CB156 etc., are enriched in *Xenopus laevis* in comparison to both low chlorine-substituted PCB congeners and high chlorine-substituted PCB congeners, such as 2 to 3-chlorine-substituted CB15+18, CB28+31 and 7 to 9-chlorine substituted CB180, CB170+190, CB200, CB171+202, CB195+208, and so on, and this may result from that medium PCBs have a higher lipophilicity than the low chlorine-substituted PCB congeners. Even medium chlorine-substituted PCB congeners are more stable in *Xenopus laevis* than both low and high chlorine-substituted PCB congeners.

PCB congeners were not distributed similarly among the samples. *Xenopus laevis* contained higher proportions of PCB compounds with middle-to-high octanol/water partition coefficients (K_{ow}), the proportion of 26 PCB congeners relative to PCBs were also assessed (Fig.5). These representative PCBs were present at levels much greater than the detection limit, and they included compounds with a wide range of K_{ow} values.

Bioconcentration factors (BCFs) for individual PCB congeners in amphibian *Xenopus laevis* 110 d habitat growing and metamorphous development are shown in Fig.5. Correlation analyses of lgBCF versus lgK_{ow} show relationships as follows:

The linear correlations of PCB congeners are as follows:

on the day 2

$$\lg BCF = -0.8236\lg K_{ow} + 7.0495 \qquad R^2 = 0.2501 \qquad (6)$$

on the day 4

$$\lg BCF = -0.5336\lg K_{ow} + 4.8897 \qquad R^2 = 0.1357 \qquad (7)$$

on the day 6

$$\lg BCF = -0.5638\lg K_{ow} + 5.3399 \qquad R^2 = 0.1471 \qquad (8)$$

on the day 80

$$\lg BCF = -0.774\lg K_{ow} + 6.8008 \qquad R^2 = 0.2435 \qquad (9)$$

on the day 95

$$\lg BCF = -0.715\lg K_{ow} + 6.6215 \qquad R^2 = 0.2224 \qquad (10)$$

on the day 110

$$\lg BCF = -0.5911\lg K_{ow} + 6.1637 \qquad R^2 = 0.1568 \qquad (11)$$

The quadratic correlations of PCB congeners are as follows:

on the day 2

$$\lg BCF = -0.5196\lg K_{ow}^2 + 6.07\lg K_{ow} - 15.687 \qquad R^2 = 0.2713 \qquad (12)$$

on the day 4

$$\lg BCF = -0.5203\lg K_{ow}^2 + 6.3691\lg K_{ow} - 17.877 \qquad R^2 = 0.1631 \qquad (13)$$

on the day 6

$$\lg BCF = -0.5196\lg K_{ow}^2 + 6.329\lg K_{ow} - 17.394 \qquad R^2 = 0.1736 \qquad (14)$$

on the day 80

$$\lg BCF = -0.4917\lg K_{ow}^2 + 5.7496\lg K_{ow} - 14.715 \qquad R^2 = 0.2643 \qquad (15)$$

on the day 95

$$\lg BCF = -0.2638\lg K_{ow}^2 + 2.7849\lg K_{ow} - 4.9216 \qquad R^2 = 0.2289 \qquad (16)$$

on the day 110

$$\lg BCF = -0.4445\lg K_{ow}^2 + 5.3053\lg K_{ow} + 13.284 \qquad R^2 = 0.1756 \qquad (17)$$

BCFs were calculated for PCB congeners and plotted against lgK_{ow} (Fig.5) to assess the influence of hydrophobicity on the concentration of *Xenopus laevis*. lgBCFs values followed a parabolic relationship with lgK_{ow}, and most values were greater than 1 in all organisms. In contrast, lgBCFs were inversely related to lgK_{ow}, and most chemicals were considerably less than equilibrium values, especially those more hydrophobic.

Correlation analysis was performed so that the relationships could be statistically quantified (Fig.5). lgBCFs were not significantly related to lgK_{ow} for PCB congeners. However, the slopes of the two correlation lines were significantly different. The PCB correlation coefficient was positive, because the PCB congener curves seemed to be parabolic. A quadratic curve was also fit to the data of PCB congeners (Fig.5), which significantly improved the accuracy of the correlation curve and was the optimum correlation function for the data. The slopes of all species-specific lgBCFs versus lgK_{ow} linear correlation functions of PCB congeners were significantly below zero (slopes of Eqs. (6)–(17)). In addition, for PCBs, the slopes of the correlation lines were significantly different among species.

Fig.4 as the typical representative shows that there is a distinct relative higher concentration in the contribution of CB77+110+154, CB118, CB138+163+164, CB105, CB90+101, CB153 during the entire experimental days, the distribution of the PCB congeners form decrease and then increase variedly accompanying with the time course. Relatively high concentrations of CB118, CB105, CB77+110+154, CB90+101, CB138+163+164 are found in *Xenopus laevis* compared to the entire 110 d time course (Fig.3). This suggests that the PCB congeners accumulate in *Xenopus laevis*, and the metabolism of these

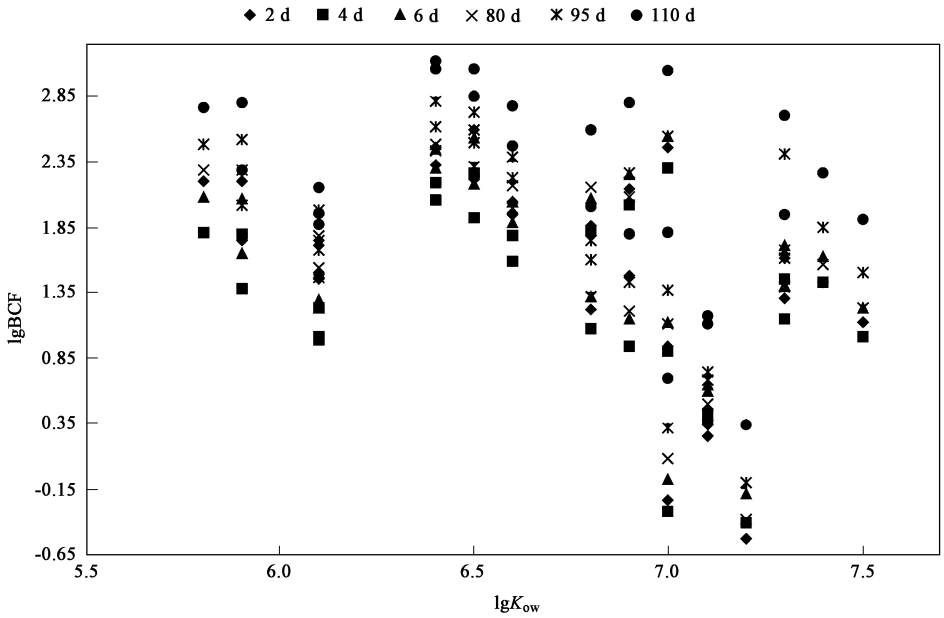

Fig. 5 Correlation analyses of lgK_{ow} versus lgBCF. The relationships between lgK_{ow} and lgBCF for individual PCB congeners on day 2, 4, 6, 80, 95, and 110.

congeners could be slow. Biotransformation of PCBs by the amphibian will be discussed in the future studies.

The decrease of PCB congeners may be due to metabolism of these congeners in *Xenopus laevis*, which was reported to occur in many fishes (Larson *et al*., 1993), mammals, and birds (Klasson *et al*., 1990; Darnerud *et al*., 1986; Murk *et al*., 1994). It is suggested that the increase of PCB congeners was due to a selective retention of these congeners in *Xenopus laevis*, to PCB congeners of substituted 5Cl and 6Cl atoms from Fig.4, CB118 and CB105 in *Xenopus laevis* of day 110 are 13.134% and 8.347%, while on day 65, the congener contributions are 10.667% and 7.598%, respectively. This typically indicates that medium chlorine-substituted PCB congeners are relatively enriched compared to both the low and high chlorine-substituted PCB congeners in the *Xenopus laevis*. However, the data regarding the bioconcentrations of CB52, CB138, CB153, CB180 showed observable differences from other author's reports that showed high level of bioconcentrations in zooplanktons, fishes, beluga whales, and so on (Gareth *et al*., 1997). The further study was to investigate the differences between amphibian *Xenopus laevis* and fishes.

3 Discussion

Amphibian *Xenopus laevis*' exposure to PCB congeners are rarely reported and assessed in the early growing life stages, although many indications have been reported that amphibian animals may represent sensitive sentinel organisms with potential efficacy in toxicological studies (Burkhart and Gardner, 1997; Cooke, 1981; Slooff, 1980). Permeable integument, complex life histories, and physiological metamorphosis add to the difficulties in measurement of the exposure of PCB congeners to amphibians (Mark *et al*., 1999). A similar result for American toads (*Bufo americanus*) was reported where dermal exposures to methoxychlor in the water contributed an order of magnitude greater to the body burdens than the oral exposures (Mark *et al*., 1999, Hall and Swineford, 1979).

The amphibian integument is a complex, vital organ for absorbing PCBs. The majority of respiration occurs through the air exchange of the skin, as much as 70% in mole salamanders (family *Ambystomidae*) to 95% in species of lungless salamanders (family *Plethodontidae*) (Mark *et al*., 1999; Phillips, 1990; Cooke, 1981; Duellman and Trueb, 1986). In small *Xenopus laevis*, it would be likely estimated that the bioconcentration of PCB congeners were derived from the systemic exposure and also result in potential effects of bioconcentrative characterization.

Because these organisms with the amphibian integument and digest pathway can uptake and concentrate high levels of PCB congeners, the results confirm that amphibian *Xenopus laevis* represent exposure and concentration of PCBs. There is the bioconcentrative lack of a great number of PCB congeners between day 55 and day 65, which was very likely due to biotransformation significantly. In particular, PCB congeners that have at least one unsubstituted lateral position are assumed to be biotransformation (Dick *et al*., 1993). In addition, a recent concentration study presented indirect evidence that fish can slowly metabolize high chlorinated PCB126 (Brown *et al*., 2002). Therefore it is not surprising that amphibians can metabolize PCB congeners. According to the evidence that was reported, the biotransformation of PCDDs usually resulted in hydrooxylated metabolites by fish (Morrison *et al*., 1996, 1998; Muir *et al*., 1986, 1988; Gobas and Schrap, 1990; Sijm and Opperhuizen, 1988; Isensee, 1978; Kleeman *et al*., 1986) which supports the assumed influence of biotransformation on bioconcentration (Dick *et al*., 1993). Freely dissolved PCBs also contribute to the body burden of mussels if the chemicals present are at high levels

in the aqueous phase (Bruner *et al*., 1994; Gewurtz *et al*., 2000). Thus, Amphibian *Xenopus laevis* accumulated significantly higher levels of PCB congeners than other organisms studied, for example, fish and bird species (Morrison *et al*., 1998; McCafferty, 1983). PCBs burdens in Amphibian *Xenopus laevis* coupled with increasing population size and fat, and are likely to cause increased body concentration within 80–110 d.

In contrast to the curves of PCB congeners between day 6 and day 12 and day 55 and day 65 were significantly lower than the other days. *Xenopus laevis* might have the capability to metabolize PCBs, and thus, degrade higher levels than other species. The assumption is consistent with Sone *et al*. (2004) report which states that the transcriptional levels of P450 aromatase and ER genes increase from stage 56 in *Xenopus laevis* (Sone *et al*., 2004; Miyashita *et al*., 2000), because the BCF is the lowest during 55–65 d. The bioconcentrative levels of PCBs in the period from day 20 to day 35 and day 80 to day 110 are similar, and it was hypothesized that significant differences in PCBs resulted from higher rates of PCB metabolism during 6–12 d and 55–65 d. Laboratory studies have found that the rates of PCB biotransformation in *Xenopus laevis* varied during this period.

The results suggest that amphibian *Xenopus laevis* probably contributed to the exposure cokinetics of PCBs during the early life stages. Metabolism was likely to be an important process contributing to the amphibian *Xenopus laevis* body burden. Because PCB levels in *Xenopus laevis* are not similar during the different development periods, significant differences of PCBs may have been due to higher rates of PCBs metabolism in *Xenopus laevis* when compared with fishes, birds, and mammals. A model developed by Thomann and Komlos (Thomann *et al*., 1999) showed that the differences between PCBs levels in crayfish were due largely to metabolism. Overall, the results indicate that amphibian *Xenopus laevis* are of a comprehensive existence of the PCBs cokinetics in the early life stages, and that *Xenopus laevis* organism would change and confine the fate of PCB congeners during this period. Contrasts in PCBs distribution during day 80 and day110 (Fig.3) demonstrated the same tendency of bioconcentration in different congener concentration. *Xenopus laevis* concentrated high proportions of the middle-to-high K_{ow} compounds, which suggests that fat was the major factor of PCBs to bioconcentration. The larger increase in concentration of PCBs from day 80 to day 110 can be explained by the course of growth and metamorphous development, and the differences between CB congeners and time courses will be discussed in the following section.

The major exposure route of the lower compounds was similar to the water phase. Amphibian *Xenopus laevis* concentrated only on most PCBs from the water. This suggests that the water and suspended particles were major take-in routes of all PCBs. The elevated proportions of the higher K_{ow} PCBs in the organisms are being bioaccumulated. Other studies demonstrated the parabolic relationship that existed between lgBCF and lgK_{ow} for PCBs (Epplett *et al*., 2000; Morrison *et al*., 1996, 1998; Hope *et al*., 1997) and the relationship also indicates that *Xenopus laevis* was selectively bioconcentrating PCB congeners. The BCF values from 1180 to 15670 of the summation of PCBs for *Xenopus laevis* in every life stage, suggest that PCBs in these stages exceeded the equilibrium predictions. The chemical disequilibria in the water were important in the concentration of PCBs in *Xenopus laevis*.

Although PCBs partition in aquatic ecosystems according to their respective hydrophobicities, their BCF are quite different due to the susceptibility of PCBs to different degradation processes. In crayfish and sunfish, Thomann and Komlos (1999) also found less-than-equilibrium PCBs BCF values, and they determined a similar inverse relationship between lgBCF and lgK_{ow}. The model they developed showed that diminished BCF with lgK_{ow} values greater than 5 resulted primarily from metabolism and decreased efficiency of chemical transfer from the gut to the organism (due to lower bioavailability sorbed to ingested sediment or prey and/or metabolism). Other factors, such as photolysis, might also have contributed to the less-than-equilibrium values.

Octanol/water partition coefficients of different PCB congeners/isomers varies no more than eightfold (Gewurtz *et al*., 2000), whereas the BCFs of the PCB congeners differ by orders of magnitude. Because the PCB congeners are differently affected by biotransformation, especially significant differences among the species-specific lgBCF versus lgK_{ow} correlation lines may be due to a variety of factors, such as differences in growth rate, lipid content, feeding preference and strategy, contaminant sources, and metabolic capabilities (Gewurtz *et al*., 2000). The present study clearly shows that the bioconcentration of PCB congeners has no relationship with their octanol/water partition coefficients, not withstanding the family of hydrophobic chemicals that often suggest that there exists a relationship (Mackay, 1982). Furthermore, there exists no relationship between octanol/water partition coefficients and biotransformation of PCB congeners as well as the family of hydrophobic chemicals.

4 Conclusions

The results of this study indicate that the concentration of PCBs exposure to the water varied due to both amphibian *Xenopus laevis* habitat growing and metamorphous development. Bioconcentration parameters (time, variable values, and BCF) for the summation of 141 PCB congeners are reported and discussed that all the congeners varied by time course. In addition, the data suggest that PCBs elimination metabolism of *Xenopus laevis* were also different during the different life stages. The PCBs metabolism was the highest during 65–75 d, higher from day 6 to day 12, and the rates of PCBs metabolism from day 30 to day 35 were lower, and from day 100 to day 110 were the lowest. Because PCBs toxicity is induced primarily by metabolites, PCB exposure is likely to be as a result of varying degrees of toxicological stress during the different developmental stages in *Xenopus laevis* in water. Amphibians also play a significant role in the

bioconcentration and metabolism comparable to fishes, birds, and mammals. This study demonstrates the special effectiveness of amphibian metamorphous development as researches of ecotoxicology. In addition, the amphibian metamorphous development is in want of fishes, birds, and mammals. *Xenopus laevis* provides information regarding bioconcentration and biotransformation levels through the whole growing course. The data demonstrate that the exposure kinetics of PCB congeners in the *Xenopus laevis* are different during early life stages, and the relationship between the octanol/water partition coefficient and the BCF is not generally applicable and that equilibrium models are not sufficient to predict chemical concentration in amphibian *Xenopus laevis* in water. Therefore, the octanol/water partition coefficient cannot be used as a predictive tool for bioconcentration for *Xenopus laevis*. Concerning amphibian *Xenopus laevis* under experimental conditions, a digital-curvilinear solution or model that incorporates different bioconcentration and metabolic processes should be plotted to predict PCB congeners behavior in the future study.

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