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Bioconcentration and Depuration of Pyribenzoxim in Common Carp (*Cyprinus carpio*)

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Pyribenzoxim (benzophenone *O*-[2,6-bis(4,6-dimethoxypyrimidin-2-yloxy)benzoyl]oxime) is a new post-emergence herbicide developed by LG Chem Investment, Ltd. (Korea) and was found to be very effective against barnyardgrass (*Echinochloa spp.*) (Koo et al. 1997), acting as a ALS (acetolactate synthase) inhibitor (Bae et al. 1997). It showed a maximal level of inhibition in whole plants within 24 hr after treatment (Koo et al. 2000). No phytotoxicity was observed and low acute toxicity (rat, oral) of >5,000 mg/kg was reported (Koo et al. 1997). The LC_{50} for common carp (*Cyprinus carpio L.*) in 96 hr was >10 mg/L. LogP measured by shake flask method is 3.04 and the solubility in water is 3.5 mg/L at 25°C (Tomlin, 2000).

The bioconcentration of certain chemicals by aquatic organisms is of great interest particularly when herbicides are used for the weed control in paddy field. Bioconcentration factor (BCF) can provide predictive information on the cumulative nature of such compound. The ethoxyresorufin *O*-deethylase (EROD) activity has been measured as a "most sensitive biological response" for assessing environmental contamination condition (Mary et al. 1993).

In this study, bioconcentration of pyribenzoxim in common carps was determined and EROD activity was monitored utilizing a flow-through exposure system for an extended period of time by performing periodic sampling, analysis of water and whole fish, and EROD assay of fish liver throughout the uptake and depuration.

MATERIALS AND METHODS

The common carp (*Cyprinus carpio L.*) was supplied by Dong Bang Agro Co. (Korea). The body length and weight of fish were 3-5 cm and 0.5-1.5 g, respectively for bioconcentration study, and 15-18 cm and 32-35 g for ethoxyresorufin *O*-deethylase assay. Feeding was done twice a day. Pyribenzoxim was not detected in the fish before exposure to test solution. Test fish were acclimated to the test water temperature for two weeks prior to test.

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Pyribenzoxim in the present study was kindly provided by LG Chem Investment, Ltd. (Korea). The purity of pyribenzoxim was more than 98.0% and was used without further purification. All solvents (HPLC grade) were purchased from Duck San Co. (Korea) while dimethyl sulfoxide (DMSO) was from Junsei Chemical Co. Ltd. (Japan). Tween 80, 7-ethoxyresorufin, and resorufin were obtained from Sigma (USA).

In a flow-through exposure system (OECD 1996; Sancho et al. 1998a), a dilution water and stock solution of pyribenzoxim were introduced into the mixing chamber by pumps (QG 400 model, USA and QG 50 model, USA), making two levels of concentration for two different tests (Table 1). After mixing, test water was continuously flowed through the test tank (20 L) with exchanging rate of 7.2 times/day. Dissolved oxygen, pH, and temperature of test water were measured at fish sampling time to ensure water environment.

Table 1. Physicochemical characteristics of test water used in study

Study	Test Concentration	D.O (mg/L)*	pH	Temp. (°C)
Bioconcentration	0.1 (mg/L)	7.2-7.5	6.74-6.78	22.5-22.8
	1.0 (mg/L)	7.0-7.4	6.96-7.08	22.8-23.2
Ethoxyresorufin *O*-deethylase	0.1 (mg/L)	7.0-7.3	6.77-6.83	22.6-23.0

*dissolved oxygen: 8.58 mg/L = 100% water saturation at 23°C

For a water recovery test, two levels of pyribenzoxim solution (0.05 ppm and 1 ppm) were made by triplicate. A certain volume (20 mL) of water was shaken with 50 mL of dichloromethane. The organic layer was evaporated under reduced pressure just to dryness at 40°C. The residue was dissolved with 2 mL of acetonitrile, and an aliquot (20 μL) was analyzed by HPLC (HP 1100 model, USA) equipped with Zorbax phenyl column (4.6 × 250 mm, 5 μm, HP, USA). Peak detection was made at 247 nm using acetonitrile: water (70:30) as a mobile phase at a flow rate of 1.0 mL/min. For a fish recovery test, a fish was treated with stock solution by triplicate to be 1 ppm and 10 ppm. A fish was homogenized with 30 mL of acetonitrile twice using a high-speed homogenizer (Heidolph DIAX 900, Germany) after the addition of 3 g of anhydrous sodium sulfate. The homogenate was filtered through a GF/A filter paper (Whatman, USA) and the filtrate was evaporated under reduced pressure just to dryness at 40°C. The residue was dissolved in 10 mL of hexane and partitioned twice with 30 mL of acetonitrile saturated with hexane. The combined acetonitrile layer was evaporated just to dryness at 40°C and the residue was dissolved with 3 mL of hexane. Florisil SPE tube (1 g/6 mL, Supelco, USA) was conditioned with 3 mL of hexane, loaded with 3 mL of sample and washed with 4 mL of 5% acetone in hexane. After washing, pyribenzoxim was eluted with 5 mL of 20% acetone in hexane. Final eluate was evaporated by N_2 gas just to dryness at 40°C. The residue was dissolved with 2 mL of acetonitrile and an aliquot (20 μL) was analyzed as in a water recovery test using a mobile phase of acetonitrile: water programmed for gradient elution follows: initial, 70:30; 0 min, 65:35; 10 min and 70:30; 16 min.

For bioconcentration study, the uptake phase test was carried out for 28 days in the high or the low concentration test with 64 fishes in each test tank, and 22 fishes for EROD assay. Depuration phase was conducted for 14 days in clean water. Water samples were taken by triplicate at the fish sampling time. Four fishes were taken for the analysis in bioconcentration study while two fishes for EROD assay. Concentration of pyribenzoxim in water or fish sample was determined using the corresponding recovery method. Bioconcentration factor (BCF) was calculated by dividing the pyribenzoxim concentration in whole fish body (mg/kg) with the pyribenzoxim concentration of test water (mg/L). The depuration rate constants (k) of pyribenzoxim from fish were calculated assuming that the depuration process follows first-order kinetics.

The fish liver microsomes were obtained according to the method of Monod and Vindimian (1991), and protein content was determined using the Bio-Rad protein reagent with bovine serum albumin as the standard (Bradford, 1976). EROD activity was measured with the method of Pohl and Fouts (1980) using the following modification (Seigfried et al. 1998). Incubation mixtures contained 150 μg protein, 0.5 mM NADPH, 10 μM dicumarol, 8 μM 7-ethoxyresorufin in 1 mL of phosphate buffer (pH 7.4). After incubation for 45 min, the reaction mixture was centrifuged at 2,000 rpm for 5 min and resorufin concentrations were measured using a fluorescence spectrometer (LS50B, Perkin Elmer, England) at 530 nm excitation and 588 nm emission.

RESULTS AND DISCUSSION

Reasonable recoveries were obtained from water and fish. Recoveries for water were 107.5 ± 2.0% (n=3) at 0.05 ppm level and 95.2 ± 3.6% (n=3) at 1.0 ppm level. Recoveries for fish were 101.3 ± 0.7% (n=3) at 1.0 ppm level and 96.7 ± 1.4% (n=3) at 10 ppm level. Standard deviation was low enough for each matrix to confirm the precision of analytical procedure at two different concentrations.

For bioconcentration study, two different concentrations of pyribenzoxim in water were selected between the 1/10 of LC_{50} value (> 10 mg/L, carp) and the value of 2.5 times of limit of detection (OECD, 1996). Therefore, the pyribenzoxim concentrations were prepared to be about 1 mg/L for the high concentration test and about 0.1 mg/L for the low concentration test. During the uptake phase, the average concentration of pyribenzxim in water were 685 ± 37 ppb for the high concentration test and 88.7 ± 2.4 ppb for the low concentration test. The coefficients of variation were 5.4% and 2.7%, respectively. These concentrations of pyribenzoxim in test water were lower than expected, probably due to the adsorption to the tubes of the experimental arrangements or the walls of the aquaria (Tsuda et al. 1993). The concentrations of pyribenzoxim in fish were gradually increased by 7 days and reached at steady-state from days 10 to days 28 in the low concentration test, and from days 17 to days 28 in the high concentration test (Figure 1).

BCFs of seven points at days 10, 14, 17, 21, 23, 26, and 28 in the low concentration test, and five points at days 17, 21, 23, 26, and 28 in the high concentration test (Table 2) were considered as the steady state (OECD, 1996). BCF for the low concentration test (BCF_L) was calculated to be 41.4 and BCF for the high concentration test (BCF_H) was 24.9, giving average BCF of 33.2.

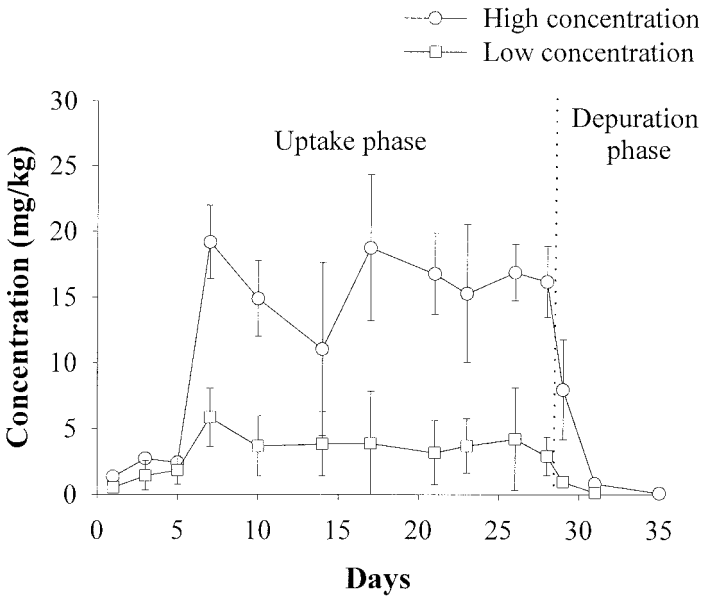

Figure 1. Changes of pyribenzoxim concentration in fish

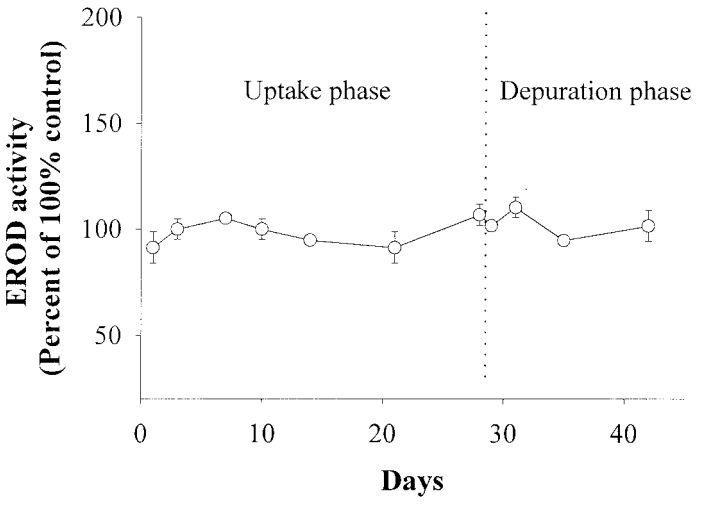

Figure 2. The changes of ethoxyresorufin *O*-deethylase activity in carp liver microsome for uptake and depuration phase.

Table 2. Bioconcentration factors of pyribenzoxim in carp

Test conc.	Bioconcentration factors in the whole carp body (n=4)										
	1*	3	5	7	10	14	17	21	23	26	28
Low	6.0	16.6	20.2	65.5	**41.2**	**44.1**	**46.5**	**36.4**	**41.1**	**47.3**	**33.2**
High	2.0	4.0	3.5	29.3	19.9	16.7	**27.3**	**26.6**	**23.4**	**22.7**	**24.6**

*exposure time (day), Boldface: steady-state

Depuration rate constant of pyribenzoxim at the low concentration test (0.97 day^{-1}) was higher than the case of the high concentration (0.72 day^{-1}) as expected. Biological half-lives of pyribenzoxim were 0.71 day and 0.96 day at the low and high concentration tests, respectively.

The concentration of pyribenzoxim in test water for EROD activity study was 85.4 ± 2.5 ppb during the uptake phase. Pyribenzoxim did not induce any significant increase in the EROD activity compare to control level (Figure 2).

The result obtained so far suggests that pyribenzoxim could be regarded as a low-bioconcentrating substance (Smrchek 1993), probably due to rapid depuration rate (Tsuda et al. 1993). Therefore, the possibility of pyribenzoxim bioconcentration is not likely to occur in the aquatic environment. The rapid depuration rate could be directly related with a fast response of the detoxification systems (Sancho et al. 1998b), resulting in no significant change of the EROD activity during the present experiment.

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