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Determination of the Bioconcentration of Phosphamidon and Profenofos in Zebrafish (*Brachydanio rerio*)

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Organophosphorus insecticides are widely used in the agriculture. Phosphamidon (2-chloro-2-diethyl carbamoyl-methyl vinyl dimethyl phosphate) and profenofos (*O*-4-bromo-2-chlorophenyl-*O*-ethyl-*S*-propyl phosphorothioate) are widely used in Korea for pest control such as white fly, rocket and plantlouse. The water solubility of phosphamidon and profenofos are 100 and 0.028 g/ℓ at 20℃ and the octanol/water partition coefficient (log Kow) are 0.8 and 1.7 (Montgomery, 1996).

Recent studies have demonstrated that organophosphorus insecticides have short and long term effect on survival of vertebrate, tissue accumulation, and on the physiological and reproductive processes of some organisms. Thus, the bioconcentration process of pesticides by aquatic organisms has been extensively studied.

The bioconcentration factor (BCF), which is generally used to estimate the propensity to accumulate chemicals in organisms, is defined as the ratio of the concentration of the chemical in whole fish at steady state to the concentration of the chemical in water during the exposure period.

On the basis of our earlier work on bioconcentration (Min, 1994; Min et al., 1994; 1995; 1996a; 1996b; Min and Cha, 1997; Min et al., 1997; 1998a; 1998b; 1998c; 1998d; Min and Cha, 1999), this paper describes the results of the bioconentration and depuration of two organophosphorus insecticides in zebrafish (*Brachydanio rerio*) at two concentration levels under flow through system (OECD, 1996).

MATERIALS AND METHODS

Phosphamidon (97% purity) and profenofos (98% purity) were obtained from Kyung Nong corporation in Korea and used without further purification. All solvents used were pesticide residue grade with no further treatment. Sep-Pak Florisil column (Waters, USA) was used for sample purification.

Zebrafish (*Brachydanio rerio*) were purchased from a commercial supplier in

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Korea, weighed 0.2 to 0.4 g and had an average length of 2.0 to 3.0 cm. All fish were acclimated in glass aquaria containing dechlorinated tap water for at least 4 weeks before use in experiments. Fish were maintained on a 8:16 hr. dark:light photoperiod. They were fed with commercial balanced fish feed at rate of 1 % body weight per day. Excrements and surplus food were removed daily. The characteristics of experimental water were: temperature, 23.5±1 ℃; pH, 7.5±0.1; DO, 7.1±0.1 ㎎/ℓ; hardness, 38±2 ㎎ $CaCO_3$/ℓ. Phosphamidon and profenofos were not detected in zebrafish before exposure to these pesticides.

A static acute toxicity test was performed according to OECD guideline 203 to determine the LC_{50} values of two pesticides (OECD, 1992). To determine the LC_{50} values, ten fish in each tank were exposed in five serial concentrations of two pesticides. The fish were not fed for 24 hr. prior to or during the acute toxicity test. The concentrations tested were 0.1, 1, 10, 50 and 100 ㎎/ℓ for phosphamidon, 2.0, 2.5, 3.0, 3.5 and 4.0 ㎎/ℓ for profenofos. Dead fish were counted and removed after every 3 hr. through 96 hr. of exposure. The LC_{50} values of each pesticide were determined using a logarithmic probability regression on actual concentrations.

Bioconcentration and depuration tests were carried out according to OECD guideline 305 in a continuous flow-through system (OECD, 1996). In the bioconcentration phase at the experiments, zebrafish were maintained at two concentrations of each pesticide for 168 hours. The stock solutions were prepared by dissolving acetone solution (2 ㎖) of phosphamidon (high exposure level 100 ㎎/ℓ, low exposure level 20 ㎎/ℓ) and profenofos (high exposure level 2 ㎎/ℓ, low exposure level 0.4 ㎎/ℓ) with dechlorinated tap water to 10 ℓ, respectively. The solutions were supplied to each of the four glass mixing chambers and connected to peristaltic pumps (Chunse BX-20, Korea) that generated constant solution flow of 3 ㎖/min diluting to the desired concentrations by constant dechlorinated tap water flow of 300 ㎖/min, the outlets were connected to each of the four 100 ℓ glass aquaria containing 250 fish. In this way, the aqueous test solution was diluted 100 times continuously. The concentrations of the pesticides in each exposure tank were [mean ± SD (n=7)] 995.4 ± 42.6 ㎍/ℓ for phosphamidon (high exposure level), 198.3 ± 17.5 ㎍/ℓ for phosphamidon (low exposure level), 19.7 ± 1.8 ㎍/ℓ for profenofos (high exposure level) and 3.9 ± 0.1 ㎍/ℓ for profenofos (low exposure level). Test aquaria renewed approximately 4.3 times a day. Zebrafish were exposed to each pesticide for 168 hours. After 6, 12, 24, 48, 72, 120, 144 and 168 hours, twenty fish were removed, rinsed with distilled water, weighed and analyzed. After the exposure period, fish were transferred to clean water with same flow-through system but without each pesticide. Twenty fish were taken at 2, 4, 8 and 12 hr, respectively.

Fish samples (ca. 5 g) were placed in a blender jar and anhydrous sodium sulfate (4 g) added. The contents were thoroughly mixed and acetonitrile (30 ㎖) was then added. The mixture was blended at high speed for 4 min. The homogenate was vacuum filtered through a GF/C glass filter. This operation

was repeated and combined filtrate was then dried in a rotary evaporator under vacuum at 40℃. The residue was redissolved in 5 mℓ of hexane and transferred to a preparative Sep-pak florisil column. The column was previously conditioned with 10 mℓ of hexane and eluted with 20 mℓ of acetone/hexane (8/2, v/v). The eluate was dried in a rotary evaporator under vacuum at 40℃ and dissolved in 2 mℓ hexane and analyzed by GC-FPD. Average recoveries (n=3) were 97% for phosphamidon and 99% for profenofos at a spiked level of 10 μg/ℓ.

To evaluate the concentration of each pesticide in the aquaria, 100 mℓ of test water were collected and extracted with 50 mℓ of ethyl ether/hexane (4/1, v/v). Extraction with ethyl ether+hexane was repeated and all extracts were combined and passed through glass column with anhydrous sodium sulfate. The eluate was dried in a rotary evaporator under vacuum at 40℃ and dissolved in 2 mℓ hexane and analyzed by GC-FPD. Average recoveries (n=3) were 88% for phosphamidon and 89% for profenofos at a spiked level of 1 μg/g. The above GC analysis was performed on a Shimadzu GC-14A with a flame photometric detector. The fused silica capillary column (DB-17, 1 μm thickness, J&W Scientifics) was 30 m by 0.53 mm ID. Nitrogen was used as carrier gas at a flow of 1 kg/cm^2. The temperature of oven, injector and detector were 230, 250 and 270℃, respectively. Quantitation was carried out by means of external standard method.

RESULTS AND DISCUSSION

The values of 24-hr LC_{50}, 48-hr LC_{50}, 72-hr LC_{50} and 96-hr LC_{50} were more than 100 mg/ℓ for phosphamidon, 2.9, 2.6, 2.2 and 2.0 mg/ℓ for profenofos, respectively (Table 1).

Table 1. Acute toxicity of phosphamidon and profenofos to zebrafish

Pesticides	LC_{50}(mg/ℓ)			
	24hr	48hr	72hr	96hr
Phosphamidon	>100	>100	>100	>100
Profenofos	2.9	2.6	2.2	2.0

Plots of bioconcentration and depuration of two pesticides are shown in Fig. 1, 2, 3 and 4. The concentration of phosphamidon in zebrafish reached an equilibrium in 12 hours at low and high concentrations. The average BCF values of phosphamidon were less than 1 at low (0.96, n=7) and high concentrations (0.89, n=7) after 12～168 hours. Depuration rate constants of phosphamidon were 0.18 h^{-1} and 0.21 h^{-1}, half-life of phosphamidon were 3.85 and 3.30 at low and high concentrations, respectively. The concentrations of phosphamidon in zebrafish at low and high concentrations rapidly decreased after 8 (0.04 μg/g) and 12 hours (0.07 μg/g).

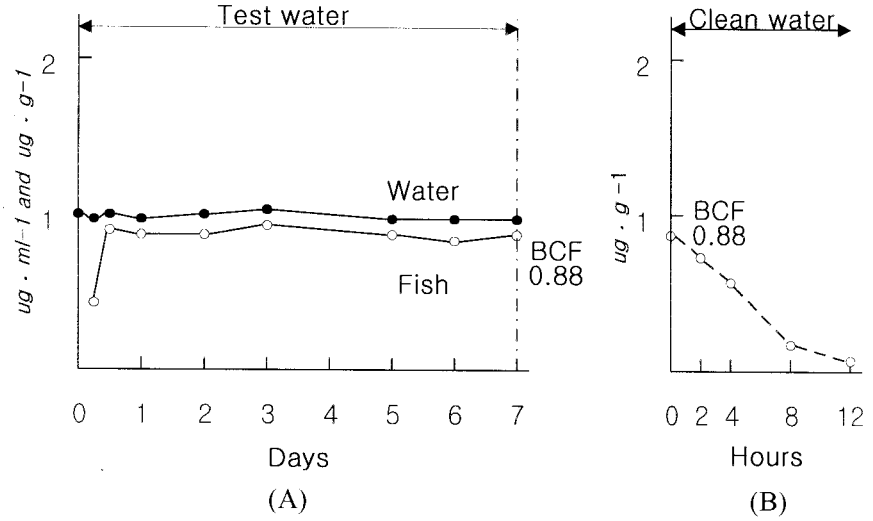

Figure 1. Intake (A) and depuration (B) of phosphamidon (high concentration) by zebrafish.

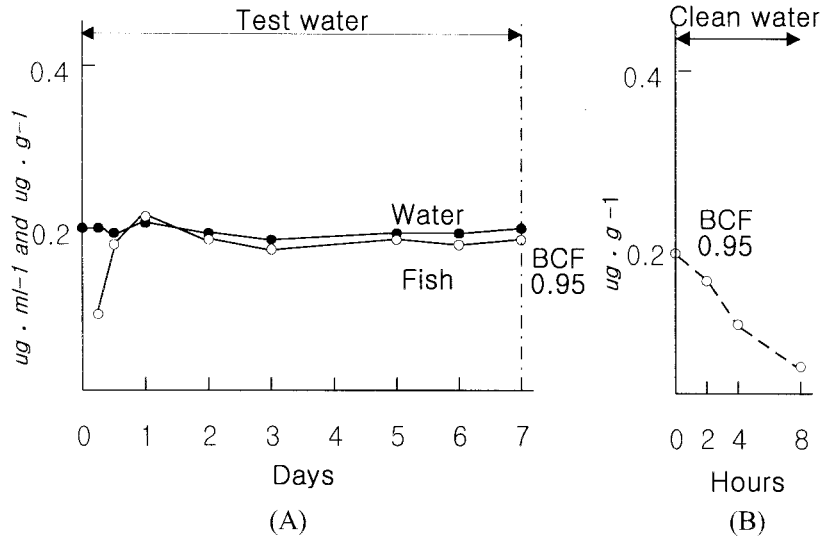

Figure 2. Intake (A) and depuration (B) of phosphamidon (low concentration) by zebrafish.

The concentration of profenofos in zebrafish reached an equilibrium in 12 hours at five-hundredth and one-hundredth concentration of 96-hrs LC_{50} (high and low concentrations). The average BCF values of profenofos were 111.3 (n=7) and 141.9 (n=7) at five-hundredth and one-hundredth concentration of 96-hrs LC_{50} (high and low concentrations) after 12 ~ 168 hours.

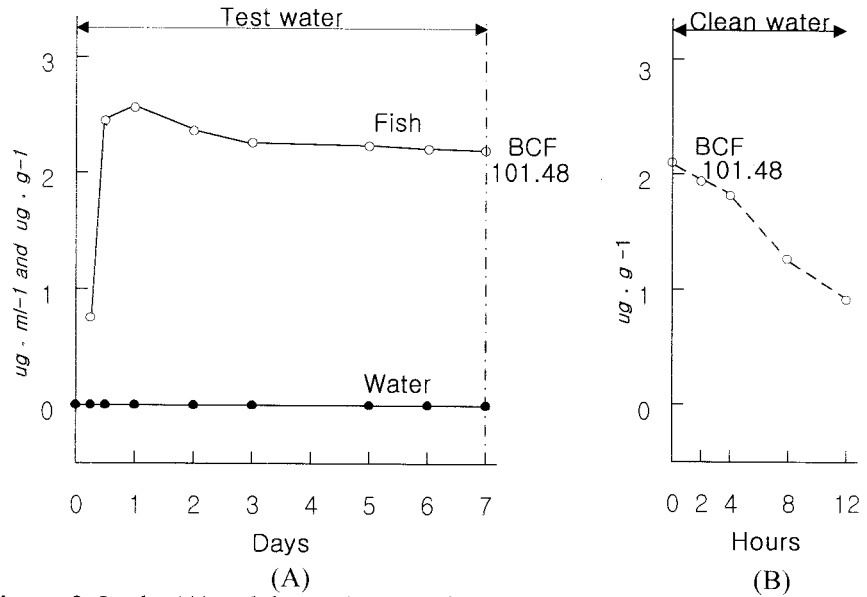

Figure 3. Intake (A) and depuration (B) of profenofos (high concentration) by zebrafish.

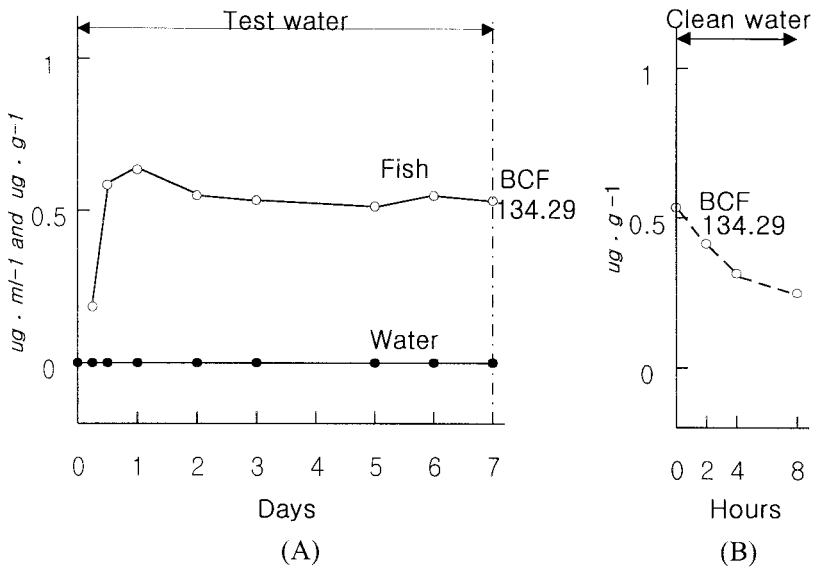

Figure 4. Intake (A) and depuration (B) of profenofos (low concentration) by zebrafish.

Depuration rate constants of profenofos were 0.10 h^{-1} and 0.09 h^{-1}, half-life of profenofos were 6.93 and 7.70 at five-hundredth and one-hundredth concentration of 96-hrs LC_{50} (high and low concentrations), respectively. The

concentrations of profenofos in zebrafish at five-hundredth and one-hundredth concentration of 96-hrs LC_{50} decreased after 8 (0.18 μg/g) and 12 hours (0.19 μg/g).

In the present study, acute toxicity of profenofos was higher than that of phosphamidon. The BCF values of profenofos were 100 times higher than those of phosphamidon, and depuration rate of phosphamidon was 2 times faster than that of profenofos.

It was suggested that the low BCF of phosphamidon was due to its very rapid depuration and very high water solubility (100 g/ℓ at 20℃), and very low lipophilicity (log K_{OW}=0.8) (Montgomery, 1996). Therefore, the bioconcentration possibility of phosphamidon is not likely to occur in the environment. The determined BCF of profenofos was much higher than that of phosphamidon. The reason is that profenofos has relatively slow depuration and low water solubility (0.028 g/ℓ at 20℃), high lipophilicity (log K_{OW}=1.7) than those of phosphamidon (Montgomery, 1996).

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