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Determination of the Bioconcentration of Methidathion and Phosalone in Zebrafish (*Brachydanio rerio*)

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Abstract: The study investigated bioconcentration of methidathion and phosalone, one of organophosphorus pesticides and the most common pesticides in Korea, on zebrafish (*Brachydanio rerio*). The acute toxicity of phosalone was higher than that of methidathion. The BCF values of phosalone were five times higher than those of methidathion, and depuration rate of methidathion was faster than that of phosalone. Only methidathion had been banned in some countries to use in agriculture areas because methidathion is known as highly toxic compound while phosalone is considered to have moderate toxicity. The results, however, of the study showed that phosalone has stronger bioconcentration effects and acute toxicity than methidathion did. Therefore, the policy for proper managements of phosalone should be urgently established.

Key words: Bioconcentration, methidathion, phosalone, depuration rate, Zebrafish.

Introduction

Water pollution has been increasing due to industrialization and civilization. Pesticides are one of the most common pollutants of aquatic ecosystems and have been known for its harmful effects on living resources. However, pesticides are the essential matter for the agriculture and various pesticides are used in Korean agricultural areas as well. Generally, pesticides are classified by the chemical compositions and structures. Synthetic organic pesticides are organochlorine pesticides, organophosphorus pesticides, carbamates pesticides etc. and the organochlorine compounds are already replaced by organophosphorus pesticides, carbamates, pyrethroids and triazines because of their long-term persistence (Montgomery, 1993). It, however, is reported that organophosphorus pesticides also have short and long term effects on ecosystems such as survival of vertebrate, tissue accumulation, and reproductivity of organisms. Thus, the bioconcentration process of pesticides by aquatic organisms has been extensively studied (El-Amrani et al., 2012; Ali-Musstjab-Akber-Shah et al., 2013; Xu et al., 2014; Zhao et al., 2014).

Among organophosphorus pesticides, methidathion [*S*-2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-

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3-ylmethyl *O,O*-dimethyl phosphorodithioate] and phosalone [*S*-5-chloro-2,3-dihydro-2-oxobenzoxazol-3-ylmethyl *O,O*-diethyl phosphorodithioate] have been widely used in Korea for pest control such as caterpillar, beetle, scale and lygus. Methidathion is a highly toxic compound, EPA toxicity class I, especially to aquatic organisms and its use has been already banned in the European Community (Gallo and Lawryk, 1991; Smith, 1993; US Public Health, 1995). Methidathion, however, is still extensively used in Korea for many crops such as fruits, vegetables, tobacco, etc. to exterminate insects and mites, although it is banned to use in rice fields. Phosalone has moderate toxicity, but it is still highly toxic to aquatic organisms. Phosalone is used as both an insecticide and acaricide for various farmlands such as nuts, citrus fruits, potatoes, etc. to control a wide range of caterpillars and beetles (Spencer, 1981; Demirin et al., 2013).

The bioconcentration factor (BCF) is the ratio of the chemical concentrations between in whole fish body at steady state and in water during the exposure period to estimate the propensity for chemical accumulations in organisms (Dimitrov, 2012; Adolfsson-Erici, 2012). The study investigated the bioconcentration and depuration rate of two organophosphorus pesticides, methidathion and phosalone, in zebrafish (*Brachydanio rerio*) at two concentration levels under flow through system (OECD, 1996).

Materials and Methods

Kyungnong corporation in Daegu, Korea provided methidathion (97% purity) and phosalone (98% purity) and those pesticides were used without further purification. All solvents were pesticide residue grade and used with no further treatment. Sep-Pak Flores column (Waters, USA) was used for sample purification.

Zebrafish (*Brachydanio rerio*), weight between 0.2 and 0.4 g, average length between 2.0 and 3.0 cm, were used. All fish were adjusted to dechlorinated tap water in glass aquaria at least four weeks before using for experiments. Fish were maintained on a 8:16 hr. dark:light photoperiod. They were fed at the rate of 1% body weight per day. Excrements and surplus food were removed daily.

The experimental water conditions were as following: temperature 23.5±1 °C, pH 7.5 ± 0.1; DO 7.1 ± 0.1 mg/*l*; hardness 37 ± 2 mg-$CaCO_3$/*l*. Methidathion and phosalone were not detected in zebrafish before exposure to the pesticides.

According to OECD guideline 203, the LC_{50} values of two pesticides were determined throughout a static acute toxicity test (OECD, 1992). Ten fish were exposed in each five different concentration of two pesticides. The serial concentrations were 15, 20, 25, 30 and 35 mg/*l* for methidathion, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/*l* for phosalone. Fish were not fed from 24 hrs. before the test to the end and dead fish were counted and removed after every 3 hr. through 96 hr. of exposure.

At the end of the test, the LC_{50} values for two pesticides were calculated using a logarithmic probability regression on actual concentrations. According to OECD guideline 305, bioconcentration and depuration tests were carried out in a continuous flow-through system (OECD, 1996). For bioconcentration phase, fish were maintained at two different concentration levels of each pesticide for 168 hours. The stock solutions were prepared by dissolving acetone solution (2 m*l*) of methidathion (exposure level 2.2 mg/*l*-22 mg/*l*) and phosalone (exposure level 0.1 mg/*l*-1 mg/*l*) with 10 *l* of dechlorinated tap water. The solution was continuously diluted 100 times and the concentrations for methidathion were 215.3 ± 15.2 μg/*l* and 21.2 ± 1.7 μg/*l*, high and low exposure level, respectively; for phosalone 11.1 ± 1.2 μg/*l* and 1.0 ± 0.1 μg/*l*, high and low exposure level, respectively [mean ± SD (*n* = 6)]. After 6, 12, 24, 48 and 72 hours, twenty fish were removed at each time and the fish were rinsed with distilled water, weighed and analyzed. After the exposure period, fish were transferred to clean water with same flow-through system. Twenty fish were taken at 6 and 12 hr, respectively and checked the pesticides concentration levels in tissues as well as in aquaria.

Results and Discussion

The values of 24-hr, 48-hr, 72-hr and 96-hr LC_{50} were 28.34, 25.98, 24.43 and 22.03 mg/ℓ for methidathion, 3.76, 2.43, 1.86 and 1.05 mg/ℓ for phosalone, respectively (Table 1). The LC_{50} value in zebrafish showed that acute toxicity of phosalone was higher than that of methidathion.

Table 1: Acute toxicity of methidathion and phosalone to zebrafish

Pesticides	*LC_{50} (mg/l)*			
	24hr	*48hr*	*72hr*	*96hr*
Methidathion	28.34	25.98	24.43	22.03
Phosalone	3.76	2.43	1.86	1.05

Plots of bioconcentration and depuration rate of two pesticides are shown in Figures 1, 2 and 3. The concentration of methidathion in zebrafish reached an equilibrium in 12 hours at one-thousandth and one-hundredth concentration of 96-hr LC_{50} (low and high concentrations). The average BCF values of methidathion were 11.25 (n = 4) and 8.72 (n = 4) after 12-72 hours.

Depuration rate constants and half-life of methidathion for both low and high concentrations were not estimated at one-thousandth and one-hundredth concentration of 96-hr LC_{50} because depuration rate of methidathion was very fast within six hours.

The concentrations of phosalone for high concentrations in zebrafish reached an equilibrium in 12 hours at one-hundredth concentration of 96-hr LC_{50}. The average BCF value of phosalone for high concentrations

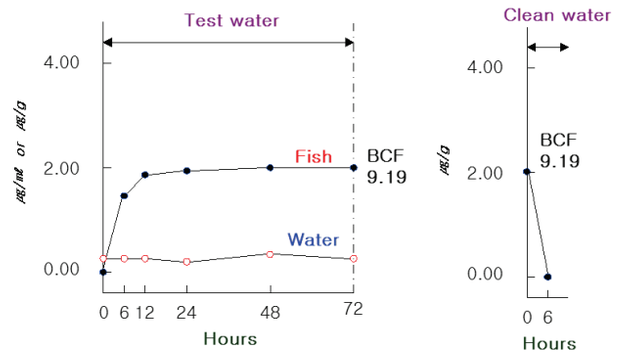

Figure 1: Intake (A) and depuration (B) of methidathion (high concentration) by zebrafish.

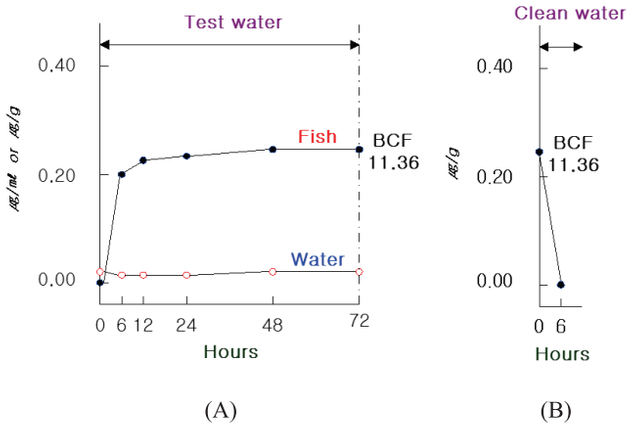

Figure 2: Intake (A) and depuration (B) of methidathion (low concentration) by zebrafish.

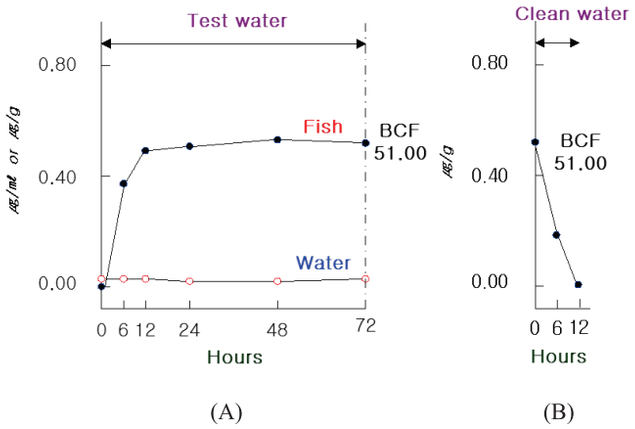

Figure 3: Intake (A) and depuration (B) of phosalone (high concentration) by zebrafish.

was 48.88 (n = 4) at one-hundredth concentration of 96-hr LC_{50} after 12-72 hours. The concentrations of phosalone in zebrafish at one-hundredth concentration of 96-hr LC_{50} decreased after six hours (0.18 µg/g). Depuration rate constant and half-life of phosalone were 0.17 h^{-1} and 4.01 at high concentrations (0.01 mg/l), respectively. However, phosalone was not detected throughout the experimental period at the low concentration (0.001 µg/ml). Depuration rate constant and half-life of phosalone were not estimated at low concentrations (0.001 mg/l) because the concentration of phosalone in zebrafish was under the detecting limit on GC.

In the present study, acute toxicity of phosalone was higher than that of methidathion. The BCF values of phosalone were five times higher than those of methidathion, and depuration rate of methidathion was faster than that of phosalone. It was considered that the low BCF of methidathion was due to its rapid depuration and high water solubility (250 mg/l at 20 °C), and low lipophilicity (log Kow = 2.22) (Montgomery, 1993). Therefore, the bioconcentration possibility of methidathion is not likely to occur in the environment. The determined BCF of phosalone was much higher than that of methidathion. The results were shown because phosalone has relatively slow depuration and low water solubility (2.6 mg/l at 20 °C), high lipophilicity (log Kow = 4.38) than those of methidathion (Montgomery, 1993).

Conclusion

The study focussed on bioconcentration of methidathion and phosalone, one of organophosphorus pesticides, since few information for bioconcentration of those two

pesticides could be available. The organophosphorus pesticides are considered to interfere the activity of acetylcholinesterase which play an important role on nerve systems and bioconcentration on those pesticides are the cause of fatal effect on human being as well as ecosystems. However, methidathion and phosalone have been still widely used. Only methidathion had been banned in some countries to use in agriculture areas; in case of Korea, it is only banned to use in rice field although it is allowed to use in other crop fields because methidathion is known as highly toxic compound while phosalone is considered to have moderate toxicity. The results showed that phosalone has stronger bioconcentration effects and acute toxicity than methidathion did. Therefore, the policy for proper management of phosalone should be urgently established.

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