Research Journal of Environmental Toxicology

ISSN 1819-3420

Academic Journals Inc.

www.academicjournals.com

Research Journal of Environmental Toxicology 8 (3): 110-116, 2014
ISSN 1819-3420 / DOI: 10.3923/rjet.2014.110.116

Effects and Bioconcentration of Dichlorvos and Phosalone on Zebrafish (*Brachydanio rerio*)

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ABSTRACT

The study investigated concentrations of dichlorvos and phosalone, the most common pesticides in Korea, in zebrafish (*Brachydanio rerio*). The bioconcentration of the pesticides reached equilibrium more rapidly in an exposure of the binary mixture compared to that in an individual exposure. The bioconcentration factor (BCF) values and depuration rate constants for zebrafish in the binary mixture (dichlorvos and phosalone) were not significantly different from those for each single pesticide. The results suggest that the bioconcentration of dichlorvos and phosalone on depuration in zebrafish can be evaluated with single pesticide's datum.

Key words: Bioconcentration, dichlorvos, phosalone, zebrafish

INTRODUCTION

Pesticides are widespread pollutants to aquatic ecosystems and by their very nature have deleterious effects on living organisms. Recently, organophosphorus pesticides, carbamates, pyrethroids and triazines have largely replaced the organochlorine compounds in the agricultural practices (Montgomery, 1996). Several studies reported that organophosphorus insecticides have short and long term effects on the survival of vertebrates, tissue accumulation and on the physiological and reproductive processes of some organisms. Thus, the bioconcentration process of pesticides and comparable chemicals by aquatic organisms has been extensively studied (Fujikawa *et al*., 2009; Jackson *et al*., 2009; Bermudez-Saldana *et al*., 2004). Among organophosphorus pesticides, dichlorvos (2,2-dichloroethenyl dimethyl phosphate) and phosalone (S-5-chloro-2,3-dihydro-2-oxobenzoxazol-3-ylmethyl O, O-diethyl phosphorodithioate) are widely used in Korea for pest controls. These substances are known as neurotoxins and also might paralyze muscles as well as respiratory systems (Fenik *et al*., 2011). Specifically for dichlorvos, the median lethal dose of oral exposure in rat is 56-80 mg kg^{-1} and that of dermal exposure is 75 mg kg^{-1} and its overdose symptoms are weakness, headache, tightness in chest, blurred vision, salivation, sweating, nausea, vomiting, diarrhea and abdominal cramps (Espeland *et al*., 2010). For phosalone, the median lethal dose of oral exposure in rat is 85 mg kg^{-1} and that of dermal exposure is 390 mg kg^{-1} and it causes toxic symptoms such as miosis, hypersalivation, hyperhidrosis, chest pressure, pulmonary edema and fecal incontinence. It is harmful, especially to aquatic creatures

(Uemura *et al.*, 2002; Jones *et al.*, 1992; Sisman, 2010) focused on dichlorvos, determining its effect on fish and few studies which reported the effect of phosalone on fish were found. No report which concerned dichlorvos and phosalone together was found. Therefore, this study focuses on those two pesticides and describes the results of the interaction of dichlorvos, phosalone or their mixtures on the bioconcentration in zebrafish (*Brachydanio rerio*) under the conditions of a flow through system (OECD, 1996).

MATERIALS AND METHODS

Dichlorvos (97% purity) and phosalone (98% purity) were obtained from Kyung Nong Corporation in Korea. Zebrafish (*Brachydanio rerio*) weighing 0.2 to 0.4 g and with an average length of 2.0 to 3.0 cm were used. All fish were acclimated in glass aquaria containing de-chlorinated tap water for at least 4 weeks (8:16 h of dark:light photoperiod) before use in experiments. The conditions of experimental water were: Temperature, 23.5±1°C; pH, 7.4±0.1; DO, 7.0±0.1 mg L^{-1}; hardness, 37±2 mg $CaCO_3$ L^{-1}.

A static acute toxicity test was performed according to OECD guideline 203 to determine the LC_{50} values of two pesticides (OECD, 1992). The concentrations of dichlorvos were 40, 50, 60, 70, 80 mg L^{-1} and those of phosalone were 1.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1}. Dead fish were counted and removed after every 3 h through 96 h of exposure. The LC_{50} values of each pesticide were determined using a logarithmic probability regression of actual concentrations.

Bioconcentration and depuration tests were carried out in a continuous flow-through system following OECD guideline 305 (OECD, 1996). Zebrafish were maintained at two concentrations of each pesticide for 72 h. The stock solutions were prepared by dissolving acetone solution (2 mL) of dichlorvos (high exposure level 55 mg L^{-1}, low exposure level 5.5 mg L^{-1}) and phosalone (high exposure level 1 mg L^{-1}, low exposure level 0.1 mg L^{-1}) with de-chlorinated tap water to 10 mL, respectively. The mixed stock solutions were prepared by dissolving acetone solution (2 mL) of dichlorvos (high exposure level 55 mL) and phosalone (high exposure level 1 mL) with de-chlorinated tap water to 10 L, respectively. The concentrations of the pesticides in each exposure tank were [Mean±SD (n = 6)] 549.3±13.2 μg L^{-1} for dichlorvos (high exposure level), 55.1±1.6 μg L^{-1} for dichlorvos (low exposure level), 11.1±1.2 μg L^{-1} for phosalone (high exposure level), 1.0±0.1 μg L^{-1} for phosalone (low exposure level), 545.7±15.3 μg L^{-1} for dichlorvos of mixtures and 10.5±1.4 μg L^{-1} for phosalone of mixtures. After 6, 12, 24, 48 and 72 h, twenty fish were removed, rinsed with distilled water, weighed and analyzed.

Fish samples (ca. 5 g) were placed in a blender jar and added 4 g of anhydrous sodium sulfate. The contents were thoroughly mixed and 30 mL of acetonitrile was then added. The mixture was blended at high speed for 4 min. The homogenate was vacuum filtered through a GF/C glass filter. The procedure was repeated and combined filtrate was then dried in a rotary evaporator under vacuum at 40°C. The residue was redissolved in 5 mL of hexane and transferred to a preparative Sep-pak florisil column. The eluate was dried in a rotary evaporator under vacuum at 40°C and dissolved in 2 mL hexane and analyzed by GC-FPD.

To evaluate the concentration of each pesticide in the aquaria, 100 mL of test water were collected and were 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 a glass column with anhydrous sodium sulfate. The eluate was dried in a rotary evaporator under vacuum at 40°C and dissolved in 2 mL hexane and analyzed by GC-FPD. Average recoveries (n=3) were 103.2% for dichlorvos and 98.7% for phosalone at a spiked level of 1 μg g^{-1}.

RESULTS AND DISCUSSION

The values of 24 h LC_{50}, 48 h LC_{50}, 72 h LC_{50} and 96 h LC_{50} were 28.34, 25.98, 24.43 and 22.03 mg L^{-1} for dichlorvos, 67.25, 61.37, 58.75 and 55.02 mg L^{-1} for phosalone, respectively (Table 1). The LC_{50} value in zebrafish showed that the acute toxicity of phosalone was higher than that of dichlorvos.

In an individual exposure of dichlorvos, the concentration of dichlorvos in zebrafish reached an equilibrium in 12 h at one-thousandth and one-hundredth concentration of the 96 h LC_{50} (low and high concentrations). The average BCF values of dichlorvos were 0.74 (n = 4, low concentrations) and 1.28 (n = 4, high concentrations) from 12-72 h (Fig. 1a, 2a). Depuration rate constants of dichlorvos were 0.16 h^{-1} and 0.13 h^{-1} and the half-lives of dichlorvos were 4.3 and 5.3 at low and high concentrations, respectively. The concentrations of dichlorvos in zebrafish at low and high concentrations rapidly decreased after 12 h (0.010 μg g^{-1}) (Fig. 1b, 2b).

In an individual exposure of phosalone, the concentrations of phosalone in zebrafish reached an equilibrium at 12 h of one-hundredth of the 96 h LC_{50} (high concentration). The average BCF value of phosalone was 48.88 (n = 4) at one-hundredth of the 97 h LC_{50} (high concentration) after 12-72 h (Fig. 3a). Depuration rate constant and half-life of phosalone were not estimated at low and high concentrations because the determination of phosalone in zebrafish was limited by the detection limit of the GC (Fig. 3b).

Table 1: Acute toxicity of dichlorvos and phosalone to zebrafish

	LC_{50} (mg L^{-1})			
Pesticides	24 h	48 h	72 h	96 h
Dichlorvos	67.25	61.37	58.75	55.02
Phosalone	3.76	2.43	1.86	1.05

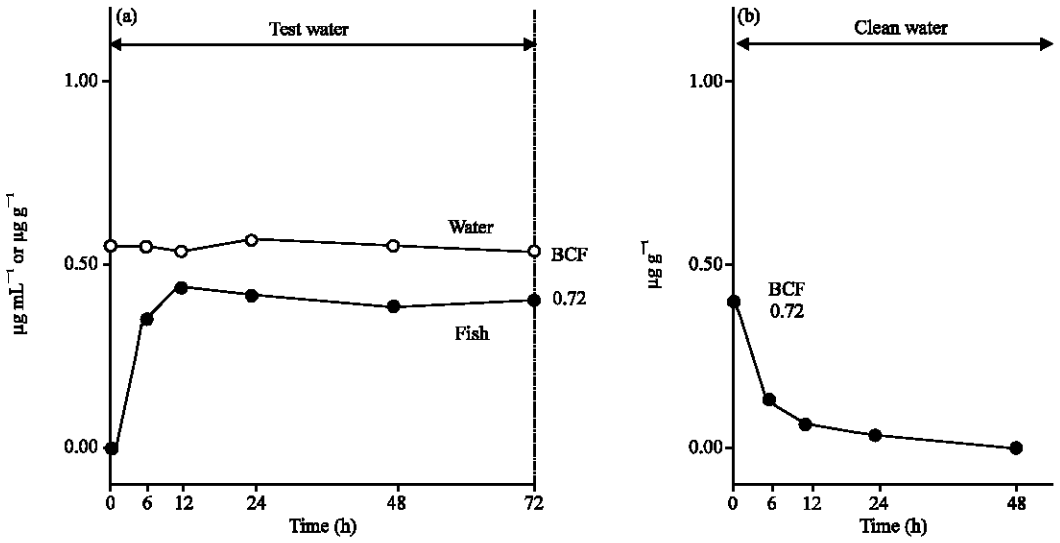

Fig. 1(a-b): (a) Uptake and (b) Depuration of dichlorvos in zebrafish exposed renewally in a flow through system to 0.55 mg mL^{-1} for 72 h followed by 48 h depuration in clean water

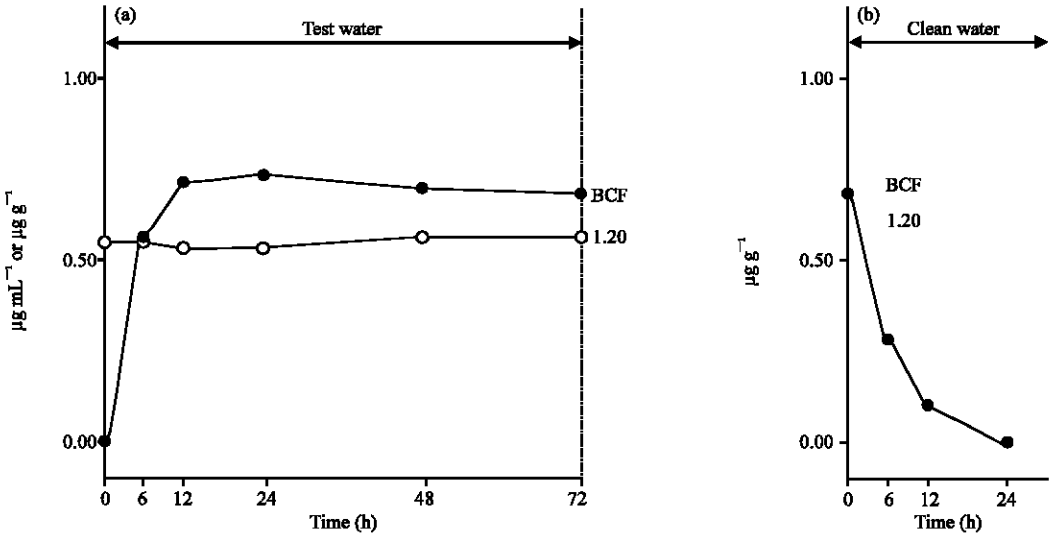

Fig. 2(a-b): (a) Uptake and (b) Depuration of dichlorvos in zebrafish exposed in a flow through system to 0.055 mg mL^{-1} for 72 h followed by 48 h depuration in clean water

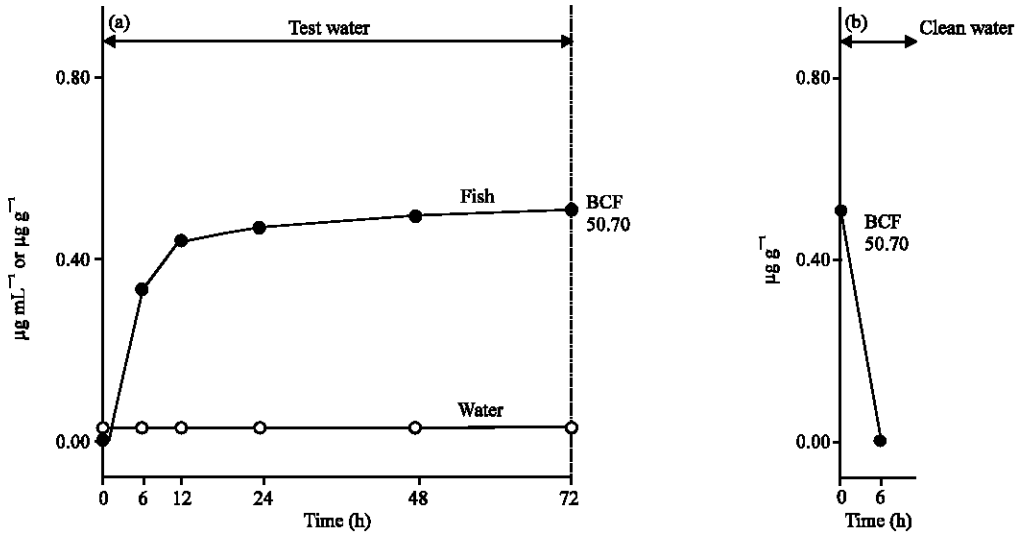

Fig. 3(a-b): (a) Uptake and (b) Depuration of phosalone in zebrafish exposed in a flow through system to 0.01 mg mL^{-1} for 72 h followed by 6 h depuration in clean water

In an exposure of the binary mixture of dichlorvos (0.55 µg mL^{-1}) and phosalone (0.01 µg mL^{-1}), the concentration of dichlorvos in zebrafish reached an equilibrium at 6 h and that of phosalone in zebrafish reached an equilibrium at 12 h. The average BCF values of dichlorvos were 0.80 (n = 5) after 6-72 h (Fig. 4a) and those of phosalone were 53.89 (n = 4) after 12-72 h (Fig. 5a). The depuration rate constant of dichlorvos was 0.12 h^{-1}, half-life of dichlorvos was 5.8, respectively. The concentrations of dichlorvos in zebrafish rapidly decreased after 24 h (0.024 µg g^{-1}) (Fig. 4b). The depuration rate constant and half-life of phosalone were not estimated because the concentration of phosalone in zebrafish was less than the detection limit of the GC (Fig. 5b).

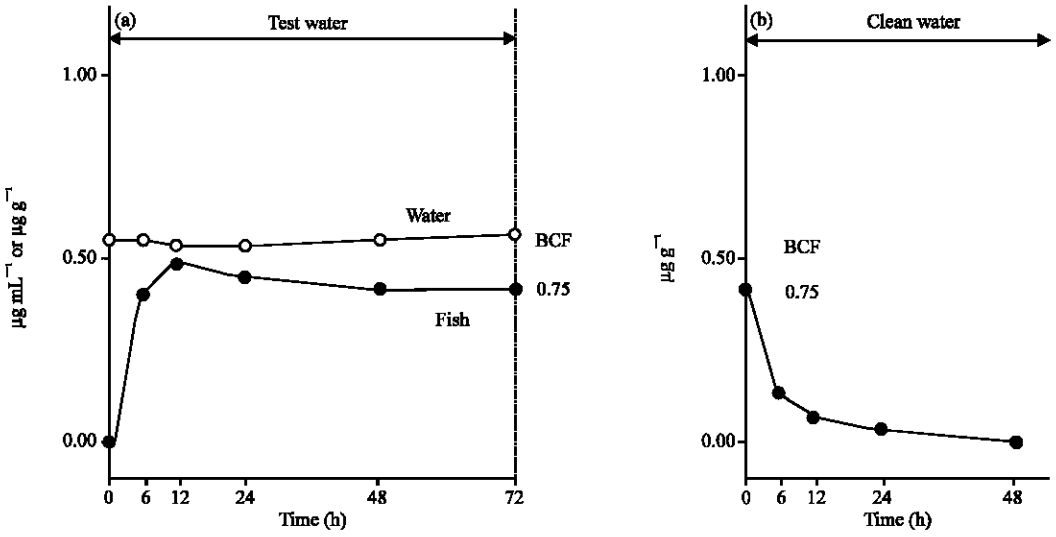

Fig. 4(a-b): (a) Uptake and (b) Depuration of dichlorvos in zebrafish exposed in a flow through system to the binary mixture of dichlorvos (0.55 mg mL^{-1}) and phosalone (0.01 mg mL^{-1}) for 72 h followed by 48 h depuration in clean water

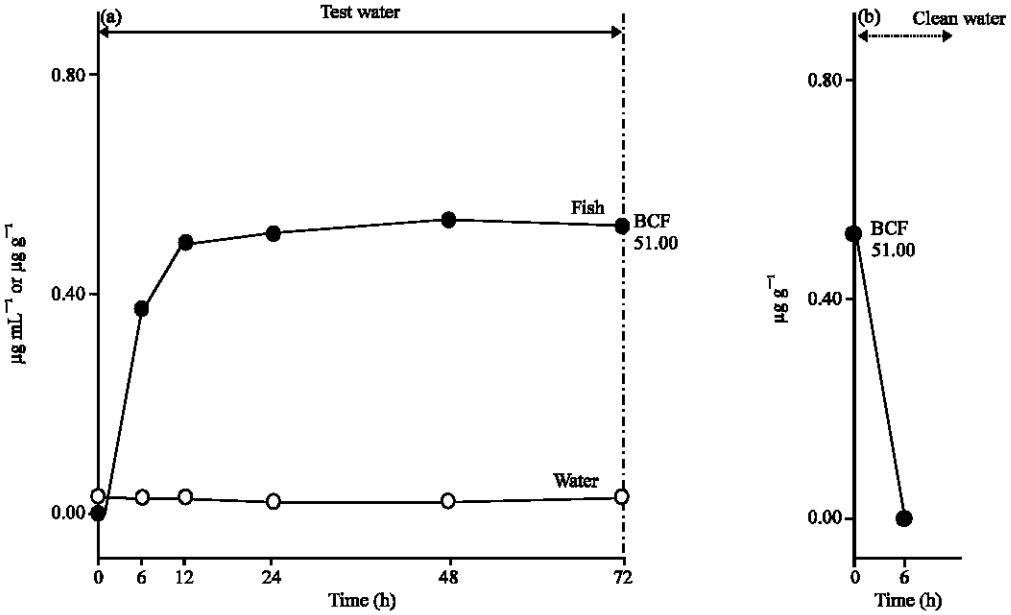

Fig. 5(a-b): (a) Uptake and (b) Depuration of phosalone in zebrafish exposed in a flow through system to the binary mixture of dichlorvos (0.55 mg mL^{-1}) and phosalone (0.01 mg mL^{-1}) for 72 h followed by 6 h depuration in clean water

In the present study, the values for BCF reached an equilibrium level more rapidly under exposure to both dichlorvos and phosalone. The average BCF values of dichlorvos (0.80, n = 5) after 6-72 h exposure and phosalone (53.89, n = 4) after 12-72 h exposure to the mixture of dichlorvos

Table 2: Summary of BCFs for dichlorvos and phosalone in zebrafish exposed to individual compounds and to the binary mixture

	Dichlorvos		Phosalone	
Exposure (h)	Single	Mixture	Single	Mixture
6	0.64±0.050	0.73±0.056	33.00±2.31	38.00±2.08
12	0.80±0.061	0.93±0.056	45.50±2.02	49.00±2.08
24	0.75±0.040	0.85±0.049	49.40±2.24	55.57±2.28
48	0.69±0.056	0.73±0.060	49.90±2.37	60.00±1.52
72	0.72±0.056	0.75±0.056	50.70±2.27	51.00±2.12

and phosalone were slightly higher than those of single dichlorvos (0.74, n = 4) or single phosalone (49.88, n = 4) after 12-72 h exposure. Therefore, the reason why the equilibrium was reached more rapidly from the mixture of dichlorvos and phosalone was likely due to an increased and competitive uptake of dichlorvos and phosalone.

Statistical analysis was carried out for each sampling time (6, 12, 24, 28 and 72 h). In statistical analysis using the t-test, the BCFs for dichlorvos and phosalone at each sampling time in the binary mixture were not significantly different from those in an individual exposure, except at 48 h ($p<0.05$) (Table 2). These results indicate that there is no influence of the binary mixture (dichlorvos and phosalone) on the BCF compared to the individual one. Also, in an exposure of the binary mixture of dichlorvos and phosalone, the depuration rate constants and half-life were not different in this study.

REFERENCES

Bermudez-Saldana, J.M., L. Escuder-Gilabert, M.J. Medina-Hernandez, R.M. Villanueva-Camanas and S. Sagrado, 2004. Modelling bioconcentration of pesticides in fish using biopartitioning micellar chromatography. J. Chromatogr. A, 1063: 153-160.

Espeland, M., M. Irestedt, K.A. Johanson, M. Akerlund, J.E. Bergh and M. Kallersjo, 2010. Dichlorvos exposure impedes extraction and amplification of DNA from insects in museum collections. Frontiers Zool., Vol. 7. 10.1186/1742-9994-7-2

Fenik, J., M. Tankiewicz and M. Biziuk, 2011. Properties and determination of pesticides in fruits and vegetables. Trends Anal. Chem., 30: 814-826.

Fujikawa, M., K. Nakao, R. Shimizu and M. Akamatsu, 2009. The usefulness of an artificial membrane accumulation index for estimation of the bioconcentration factor of organophosphorus pesticides. Chemosphere, 74: 751-757.

Jackson, S.H., C.E. Cowan-Ellsberry and G. Thomas, 2009. Use of quantitative structural analysis to predict fish bioconcentration factors for pesticides. J. Agric. Food Chem., 57: 958-967.

Jones, M.W., C. Smooerville and R. Wootten, 1992. Reduced sensitivity of the salmon lous, *Lepeophtheirus salmonis*, to the organophosphate dichlorvos. J. Fish Dis., 15: 197-202.

Montgomery, J.H., 1996. Groundwater Chemicals. 2nd Edn., Lewis Publishers, Boca Raton.

OECD, 1992. OECD guideline for testing of chemicals, t est no. 203: Fish, acute toxicity test. OECD, Paris, France. http://www.oecd.org/chemicalsafety/risk-assessment/1948241.pdf

OECD, 1996. OECD guidelines for testing of chemicals, proposal for updating guideline 305: Bioconcentration: Flow-through fish test. OECD, Paris, France. http://www.epa.gov/scipoly/sap/meetings/2008/october/305_bioconcentration_flowthrough_fish_test.pdf

Sisman, T., 2010. Dichlorvos-induced developmental toxicity in Zebrafish. Toxicol. Ind. Health, 26: 567-573.

Uemura, S.H., H. Kawamura, M. Tsuji, S. Tomita and S. Maeda, 2002. Pesticide Toxicity Dictionary. 2nd Edn., Three Star Hall, New York.