Bull Environ Contam Toxicol (2011) 86:596–600
DOI 10.1007/s00128-011-0281-4

Influence of Bioassay Volume, Water Column Height, and Octanol–Water Partition Coefficient on the Toxicity of Pesticides to Rainbow Trout

Ilhan Altinok · Erol Capkin · Halis Boran

Received: 3 September 2010 / Accepted: 15 April 2011 / Published online: 29 April 2011

Abstract Effects of water volume and water column height on toxicity of cypermethrin, carbaryl, dichlorvos, tetradifon, maneb, captan, carbosulfan endosulfan and $HgCl_2$ to juvenile rainbow trout (*Oncorhynchus mykiss*, 3.2 ± 0.7 g) were evaluated in different glass aquaria under static conditions. When fish were exposed to the chemical compounds in 23 cm water column height (25 L), their mortality ranged between 0% and 58%. At the same water volume, but lower water column height (9 cm), mortality of fish increased significantly and was in a range from 60% to 95%. At the same water column height, toxic effects of chemicals were significantly higher in 25 L water volume than that of 8.5 L, water except maneb which has lowest (−0.45) octanol–water partition coefficient value. Mortality rates ratio of 9 and 23 cm water column height ranged between 1.12 and 90 while mortality rates ratio of 9 and 25 L water volume ranged between 1.20 and 4.0. Because actual exposure concentrations were not affected by either water volume or water column height, we propose that increased pesticides' toxicity was related to an increase in bioassay volume, since more pesticide molecules were able to interact with or accumulate the fish. However, there seem to be no relationship between the effects of water volume, water column height and Kow value of chemicals with regard to toxicity in juvenile rainbow trout.

Keywords Water volume · Water column height · Rainbow trout · Pesticides · Octanol–water partition coefficient

I. Altinok (✉) · E. Capkin · H. Boran
Faculty of Marine Science, Department of Fisheries Technology Engineering, Karadeniz Technical University, Surmene, 61530 Trabzon, Turkey
e-mail: ialtinok@gmail.com

Aquatic toxicity tests or bioassays are routinely employed to predict the environmental impact of different toxic chemicals on aquatic organisms. When toxicity tests are repeated in different laboratories, toxicity data elicited from these bioassays is the lack of uniformity between results. Although a small part of such variation comes from random factors, large portion of the variations often cannot be explained (Sprague 1995). Because of various characteristics of water and of organisms, sometimes these differences between laboratories in LC_{50} may approach a factor of 10 (Fogels and Sprague 1977).

The octanol–water partition coefficient (Kow) is the ratio of the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature (Finizio et al. 1997). Organic compounds of Kow have been used become of paramount importance in predictive environmental studies. Kow is used in evaluative models for the prediction of distribution among environmental compartments (Mackay 1991) in equations for estimating bioaccumulation in fish (Veith et al. 1979).

In the toxicity studies in our laboratory, sometime different sizes of aquarium were used in the same toxicity test and different LC_{50} values were obtained although fish and water characteristics were the same. These differences would be a result of aquarium sizes and water column height. Even though effects of biotic and abiotic characteristics such as test species, life stage, size, nutrition, health, parasitism, acclimation, water temperature, pH, salinity, suspended solids, hardness, and alkalinity on toxicity of pesticides well documented (Sprague 1995; Altinok et al. 2006; Capkin et al. 2006), no research has been done to test effect of bioassay volume and water column height on toxicity of pesticides on fish or vertebrates. Only one research dealt with the importance of bioassay volume in toxicity tests using algae and aquatic invertebrates (Stratton and Giles 1990). Therefore, in the present study, effects of bioassay volume in toxicity of rainbow trout exposed to various pesticides and $HgCl_2$ were tested.

Materials and Methods

Juvenile rainbow trout *Oncorhynchus mykiss* (2.2 ± 0.7 g; 5.1 ± 0.4 cm; mean ± SD) were obtained from Karadeniz Technical University, Faculty of Marine Sciences and held in 200 L closed recirculating systems for 3 weeks to acclimate to laboratory conditions prior to experiments. During the acclimation period, about 20% of the water in recirculating system was replaced daily. Throughout the acclimation period and subsequent periods of pesticides exposure, fish were held under a photoperiod of 12 h light:12 h dark. During acclimation, fish were fed 5% body weight twice a day with commercial trout pellets. Fish were not fed 48 h before exposure and during the toxicity tests.

During exposure to pesticides, water quality characteristics (temperature, pH, total hardness, alkalinity, unionized ammonia, nitrite, and dissolved oxygen) and pesticide concentration in each treatment were measured daily. Total ammonia was measured by an indophenol method, and nitrite was measured by an azo method. Total hardness and total alkalinity were measured by titration method. Dissolved oxygen concentration was measured by Winkler method (Boyd and Tucker 1992). Water temperature and pH were determined with a glass electrode (Thermo Orion, Beverly, MA, USA).

Experiment was conducted based on description Altinok et al. (2006). Briefly, the fish were examined and determined to be free of external parasites before the exposure. After acclimation, fish from one of the acclimation tanks were randomly transferred to 1 of 32 glass aquaria containing different volume and height of water column (Table 1). Quadruplicate random groups of 10 fish each were subjected to experimentation for 96 h in static freshwater containing different actual concentration of pesticides (Table 2). Doses selected were based on 96 h LC_{50} values of pesticides assessed earlier in the laboratory (unpublished data). Test solutions of pesticides were either dissolved in or mixed with 250 mL distilled water and then added in aquaria. Control tanks received 250 mL distilled water. This study was conducted under OECD Guideline No. 203 under static-renewal test conditions (OECD 1992). Fifty percent of the test solution was renewed each day. During the 96 h acute toxicity experiment, water in each aquarium was aerated and had the following characteristics: dissolved oxygen 7.87 ± 0.21 mg/L, temperature 17.4 ± 0.33°C, pH 7.58 ± 0.19, total hardness 32.2 ± 1.90 mg/L as $CaCO_3$, alkalinity 26.12 ± 2.4 mg/L as $CaCO_3$, unionized ammonia 8 ± 2 mg/L and nitrite 6.4 ± 3.9 μg/L. Fish were considered dead when gill opercula and body movement ceased, and they were removed immediately (Altinok et al. 2006; Capkin et al. 2006).

To test effect of bioassay volume and height of water column on toxicity of pesticides to fish, Kaplan–Meier survival and failure time analysis tests (KMSFTAT) were used. After analyzing survival data with KMSFTAT, when significant differences were found ($p < 0.05$), comparisons among means were made with a Cox–Mantel test (Statistica, Statsoft Inc., Tulsa, OK, USA). A single multiple regressions (e.g., mortality vs. Kow, water volume, water column height) was performed with mortality serving as the dependent variable and Kow, water volume, and water column height serving as independent variables.

Results and Discussion

No fish died during the acclimation period before pesticides exposure, and no control fish died during toxicity tests. No behavioral changes were observed between water volume treatments or height of water column treatments. Although water volume was the same (25 L) and each aquaria contained same number of fish, toxicity of cypermethrin, carbaryl, dichlorvos, captan, carbosulfan, endosulfan, tetradifon, and mercuric chloride was significantly affected by height of water column (9 and 23 cm) which did not affect maneb toxicity (Fig. 1).

Differences in LC_{50} due to the changes in water volume or water height should be whether the animals are stressed due to crowded conditions and adsorption of contaminants to the container wall. In the present study, fish stocking rate was 1 g/L which was less than OECD Guideline recommendation under static-renewal test conditions (OECD 1992); therefore, being stressed because of crowded condition is not possible in the present study. In the present study, water samples were analyzed for chemical concentrations and their concentrations were the same between different water volume and different water height. Since it is important to measure analytically how these chemical concentrations change over time in different water volumes or heights, and these would have effect on the actual exposure concentration by the organisms, actual exposure concentrations of chemicals were measured and concentration of them did not affected by water volume or water height. Differences in LC_{50} due to the changes in water volume or water height should be affected by water volume and water height.

OECD recommends maximum loading of 1.0 g fish/L for static and semi-static tests (OECD 1992). In the present

Table 1 Experimental parameters

	Water volume (L)	Water column height (cm)	Aquarium size (cm^3)
Experiment I	25	9	65 × 45 × 35
	9	9	40 × 30 × 45
Experiment II	25	9	65 × 45 × 35
	25	23	40 × 30 × 45

study, using average fish weight (2.2 g/fish) and 10 fish/treatment, the 9 L treatment contained about 2.4 g/L (22 g fish/9 L test solution) and the 25 L treatment contained about 1.3 g/L (22 g fish/25 L test solution), exceeding the maximal loading of 1.0 g/L recommended by OECD. Therefore it should be expected that mortality of fish is higher in 9 L aquaria than that of 25 L aquaria because of stocking density but the result is completely opposite maybe because of more pesticides available in higher volume of aquaria to interact with or accumulate the fish. Concentrations of chemicals were checked every day and chemicals concentrations were not changed before and after the experiments because 50% of the test water was changed every day. Therefore chemical concentrations were the same for all experiment.

Table 2 Active ingredient, commercial names, and target organism group of pesticides and their concentrations used in the present study

Active ingredient of pesticides (chemical group)	Commercial names of pesticides	Target organism	Actual concentration used in the present study	Method used to analyze and references
Cypermethrin (200 g/L) (Pyrethroid)	Arrivo 20 EC (Hektaş, Gebze-Kocaeli, Turkey)	Insecticide	80 ± 2.2 μg/L	GC-ECD (Brito et al. 2002)
Carbaryl (85%) (Carbamate)	Carvil 85 WP (Safa Tarim, Konya, Turkey)	Insecticide	1.42 ± 0.11 mg/L	LC (UV detector) (Irth et al. 1990)
Dichlorvos (550 g/L) (Organophosphorus)	Safa DDVP 550 EC (Safa Tarim, Konya, Turkey)	Insecticide	0.30 ± 0.03 mg/L	LC-DAD (Irth et al. 1990)
Tetradifon (75.2 g/L) (Organochloride)	Teterafon EC (Hektaş, Gebze-Kocaeli, Turkey)	Acaricide	15 ± 1.8 μg/L	GC–ECD (Brito et al. 2002)
Maneb (80%) (Dithiocarbamate)	Saneb M-22 (Safa Tarim, Konya, Turkey)	Fungicide	10.8 ± 1.4 mg/L	HPLC (UV detector) (Barcelo and Hennion 2003)
Captan (50%) (Dicarbotimite)	Captan (Hektaş, Gebze- Kocaeli, Turkey)	Fungicide	0.2 ± 0.01 mg/L	GC-ECD (Brito et al. 2002)
Carbosulfan (250 g/L) (Carbamate)	Altin Amiral 25 EC (Agro Altin, Kutlukent-Samsun, Turkey)	Insecticide	100 ± 5.2 μg/L	LC (UV detector) (Irth et al. 1990)
Endosulfan (32.9%) (Organochloride)	Thiodan 35 WP (Bayer Crop Science AG, Rhein Germany)	Insecticide	1.6 ± 0.05 mg/L	GC-ECD (Brito et al. 2002)
Mercuric chloride	$HgCl_2$ (Sigma–Aldrich, St. Louis, MO, US)	Fungicide	0.72 ± 0.01 mg/L	AAS (Weltz and Schubert-Jacobs 1991)

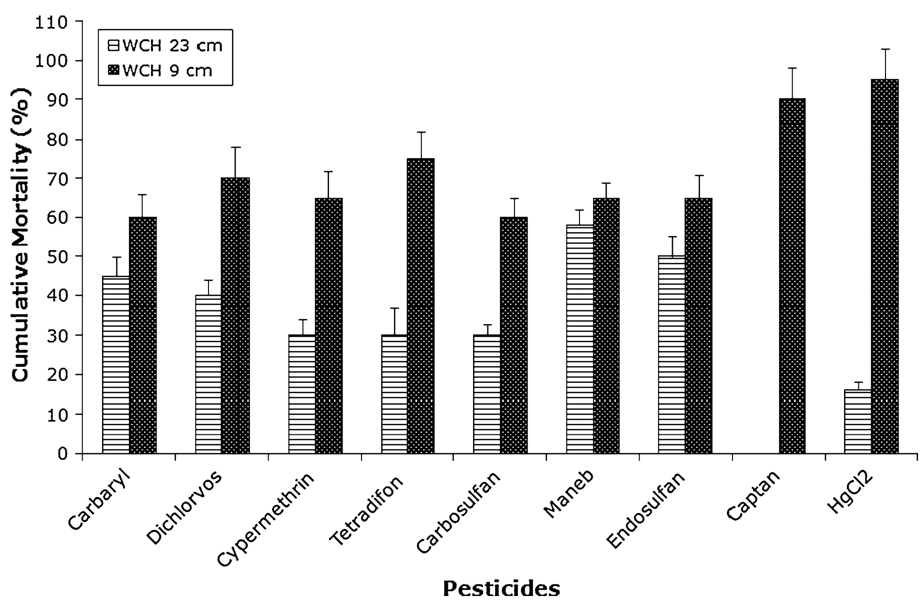

Fig. 1 Effects of water volume ($V = 9$ L and $V = 25$ L) on toxicity of pesticides when water column height (WCH = 9 cm) was same. Water volume was significantly affected pesticides toxicity except maneb toxicity. *Error bars* are standard error

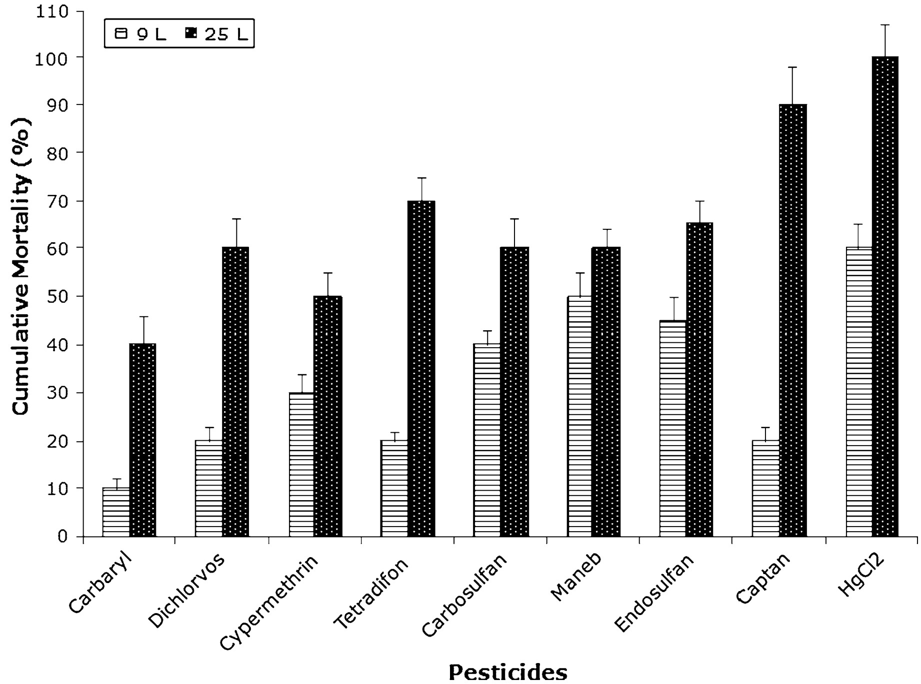

Fig. 2 Effects of water column height (WCH = 9 and 23 cm) on toxicity of pesticides when water volume (V = 25 L) was same. WCH was significantly affected pesticides toxicity except maneb toxicity. *Error bars* are standard error

Table 3 Mortality rate ratios of water column heights and water volumes

Active ingredient of pesticides	Mortality rates ratio of 9 and 23 cm water column height	Mortality rates ratio of 9 and 25 L water volume	Octanol water partition coefficient at 20°C	References
Cypermethrin	2.17	1.67	6.30	IPCS (2001)
Carbaryl	1.33	4.00	2.36	USEPA (1984)
Dichlorvos	1.75	3.00	1.16	ATSDR (1997)
Tetradifon	2.50	3.50	4.61	PPED (2011)
Maneb	1.12	1.20	−0.45	PPED (2010)
Captan	90.0	4.50	2.35–2.54	USEPA (1984)
Carbosulfan	2.00	1.50	7.42	PPED (1988)
Endosulfan	1.30	1.44	3.55–3.62	ICSC (1993)
Mercuric chloride	5.94	1.67	0.10	IPCS (2003)

The most affected pesticide was captan by a factor of 90. Toxicity of pesticides, except maneb, was significantly affected by different bioassay volume (9 and 25 L) with the same water column height (9 cm). Only increasing water volume with the same height of water column can also decrease toxicity of pesticides (Fig. 2) may be due to water pressure or Octanol–Water Partition Coefficient (Kow) of chemicals because only maneb has −0.45 Kow value (Table 3). At the same water column height, toxicity of carbaryl, dichlorvos, tetradifon, maneb, and endosulfan was much more affected by water volume than that of water column height at the same water volume (Table 3). Therefore, mortality of fish caused by pesticides can be reduced by reducing water volume and increasing water column height.

Pesticides toxicity can be decreased by decreasing the bioassay volume with the same water column height or by reducing surface area with the same water volume (i.e., increasing water column height). On the other hand, Kow is used in many environmental studies to help determine the fate of chemicals in the environment. An example would be using the coefficient to predict the extent a contaminant will bioaccumulate in fish (Chiou 2002). In the present study, increase pesticides toxicity was reflected by an increase bioassay volume, since more pesticides was available to interact with or accumulate the fish. Another word, toxicity of pesticides was significantly decreased with increasing water column height maybe due to fish physiology that could be affected by height of water column or behavior of chemical on the water column.

In the present study, biotic (test species, life stage, size, nutrition, health, parasitism, and acclimation) characteristics of rainbow trout and abiotic (water temperature, pH, salinity, suspended solids, hardness, and alkalinity) characteristics of water were the same between toxicity tests and only the differences were on water volume or height of water column. Therefore, factors that modify toxicity or differences in toxicity of pesticides only came from water volume or height of water column. Based on regression analyses, there was no relationship between Kow of chemicals and water volume or water column height on the toxicity of fish.

In conclusion, (1) toxicity (mortality) is increased when water volume is increased at the same water column height (Fig. 1), (2) toxicity is increased when water column height is decreased at the same water volume (Fig. 2), and (3) toxicity is unrelated to Kow (Table 3, Figs. 1, 2) except maneb.

Acknowledgment The study was supported by Karadeniz Technical University Research Fund and Faculty of Marine Science.

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