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Acute Toxicity of Dichlorvos to *Branchiura sowerbyi* (Beddard, 1982)

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ABSTRACT

In the present study acute toxic effects of Dichlorvos to benthic oligochaete worm, Branchiura sowerbyi were evaluated. The 24, 48, 72 and 96 h LC50 values of Dichlorvos to B. sowerbyi were 11.83, 10.38, 8.24 and 7.15 mg/l respectively. The mortality rate of the B. sowerbyi varied significantly (p<0.05) with increasing concentration of test chemical at all exposure times. The mortality rate of the worm also varied significantly (p<0.05) at all the exposure times (24, 48 and 72 and 96h) at 7.5 mg/l of Dichlorvos and above. The worms exposed to test chemical gradually reduced their clumping tendency and movement with increasing concentrations at 7.5 mg/l and above during 72 and 96h of exposure times.

KEYWORDS : Dichlorvos, LC50, *Branchiura sowerbyi*, acute toxicity.

Introduction

Dichlorvos ($C_4H_7C_{12}O_4P$), an organophosphate pesticide is commonly used as dust, granules and emulsion for controlling variety of insect pests and mites of plants and farm animals (Binukumar and Gill, 2010; Das, 2013; Deka and Mahanta, 2015). It is also used as antihelminthic agent (URL-1). It quickly degrades in nature (EPA, 2006). It is a contact and stomach poison and becomes highly toxic when it enters into the animal body through inhalation, swallowing or absorption by skin (Musa et al., 2010). It inhibits acetyl-cholinesterase (AChE) at synapse where acetylcholine is accumulated and leads to continuous triggering of the central nervous system of the target and non-target animals causing death (Binukumar and Gill, 2010; Das, 2013; IARC-WHO, 1991). Several reports on the toxicity of Dichlorvos to fish and other aquatic animals through impairment of metabolism are also available (Suntio et al., 1988; Das, 2013).There are several reports on the acute toxicity of Dichlorvos to fish. The 96h LC_{50} values ranged between 0.004-11.6 mg/l (Shepard, 1986; Velmurugan et al., 2009; Günde and Yerli, 2012; Bhat et al., 2012 and Ahmad and Gautam, 2014). The report on acute toxicity of Dichlorvos to *Branchiura sowerbyi* is almost absent. This scenario encouraged us to evaluate the toxic effects of Dichlorvos to *Branchiura sowerbyi* that plays a very important role in the detritus food chain in a natural water body as it serves as the food of fish. In the present study the acute toxic effects of Dichlorvos to benthic oligochaete worm, *Branchiura sowerbyi* were evaluated.

Materials and Methods

The test chemical was commercial grade Dichlorvos (76% EC). It was collected from the local market. The test organisms used in this static bioassay were the benthic oligochaete worms, *Branchiura sowerbyi* (Class: Oligochaeta, Family: Tubificidae). The tubificid worms were collected from local market and were allowed to acclimatize in the laboratory condition for 72h before the experiment.

The static replacement bioassays with the test organism, *Branchiura sowerbyi* were conducted in 400 ml Borosil glass beakers each containing 250 ml un-chlorinated tap water. The physico-chemical properties of test water were as follows: temperature 28.0 ± 0.5°C, pH 7.4 ± 0.5, free CO_2 17.3 ± 1.5 mg/l, dissolved oxygen 5.6 ± 0.7 mg/l, total alkalinity 160 ± 5.0 mg/l as $CaCO_3$, hardness 115 ± 4.3 mg/l as $CaCO_3$. The acute toxicity test was conducted with four replicates for each concentration of Dichlorvos along with sufficient control.

Initially the rough range finding tests of Dichlorvos to *Branchiura sowerbyi* were conducted to determine the range of concentrations of the test chemical at which the mortality of the test organisms occur. The selected test concentrations of toxicant were finally used to determine the 24, 48, 72 and 96h LC_{50} values of Dichlorvos to *Branchiura sowerbyi*. Each beaker contains ten test organisms with the mean length of 10.6 ± 0.5 mm. During the experiment, the number of dead organisms was counted at every 24h of exposure. The dead organisms were removed immediately to avoid any organic decomposition. A specific amount of water was replaced every 24h by un-chlorinated stock water and a certain quantity of Dichlorvos was added immediately to test medium to make a desired concentration. The bioassays as well as the estimation of all the physico-chemical parameters of the test water were done following the methods of APHA (2012). The behavioural changes of treated worms were also studied during the experiment following the methods of Mukherjee & Saha (2012) and Bej et al., -(2015). The LC_{50} values of 24, 48, 72 and 96h along with 95% confidence limits were calculated with the help of a statistical computer software program (US EPA, 1999). The obtained data of percent mortality of the *Branchiura sowerbyi* were subjected to ANOVA using the R- software (R Development Core Team, 2011) followed by DMRT to determine the significant variation among means at different concentrations and times of exposure.

Results and Discussion

The 24, 48, 72 and 96 h LC_{50} values of Dichlorvos to *B. sowerbyi* were 11.83, 10.38, 8.24 and 7.15 mg/l respectively (Table 1). No mortality of worm was recorded in control during the experiment. The mortality rate of the *B. sowerbyi* varied significantly ($p<0.05$) with increasing concentration of Dichlorvos at all exposure times (Table 2). The mortality rate of the worm also varied significantly ($p<0.05$) over the control at all the exposure times (24, 48 and 72 and 96h) at 7.5 mg/l of test chemical and above concentrations (Table 2).

Branchiura sowerbyi showed different unusual behaviours with the increasing concentration of Dichlorvos and the progress of time of exposure. The control worm showed clumping tendency and normal movement throughout the experiment, but the worms exposed to test chemical gradually reduced their clumping tendency and movement with increasing concentrations (7.5 mg/l and above) at 72 and 96h of exposures. The worms become sluggish at these doses.

In the present study the 96h LC_{50} value of Dichlorvos to *Branchiura sowerbyi* corresponds with a few earlier findings to *Cirrhinus mrigala* (9.1 mg/l), *Clarias batrachus* (8.9 mg/l), Sheephead minnow (7.5 mg/l) (Verma et al., 1983; Jones and Devis, 1994; Velmurugan et al., 2009). On the other hand the present 96h LC_{50} value of Dichlorvos to *Branchiura sowerbyi* is much higher than the 96h LC_{50} values of Dichlorvos to many other fish like *Salvelinus namaycush* (0.2 mg/l), *Cyprinus carpio* (2.51 mg/l), *Mystus vittatus* (0.5 mg/l), *Lepomis macrochirus* (0.48 mg/l), (Johnson and Finley, 1980; Verma et al., 1980, 1981; Mayer and Ellersieck, 1986; Günde and Yerli, 2012). The 48h LC_{50} value of Dichlorvos to *B. sowerbyi* is also higher than the 48h LC_{50} value of Bluegill Sunfish (0.7 mg/l) and Rainbow trout (0.5 mg/l) (Anon, 1968; Pimentel, 1971). The above findings indicate that *Branchiura sowerbyi* is comparatively less susceptible to Dichlorvos than fish.

The acute toxicity data (LC_{50} values) of Dichlorvos to *Branchiura sowerbyi* can be used in the determination of its safe level before the release of agricultural waste containing Dichlorvos to the natural water bodies.

Table 1: LC_{50} values along with 95% confidence limits of Dichlorvos to the *Branchiura sowerbyi* at different hours of exposure (24, 48, 72 and 96h)

Test organism	Concentration of Dichlorvos (mg/l)			
	24h	48h	72h	96h
Branchiura sowerbyi	11.83 (10.62-13.11)	10.38 (9.27-11.40)	8.24 (7.04-9.44)	7.15 (6.13-8.09)

Table 2: Mean values (± SD) of % mortality of *Branchiura sowerbyi* exposed to different concentrations of Dichlorvos at different times of exposure (24, 48, 72 and 96h). Mean values within columns indicated by different superscript letters (a-h) and mean values within rows indicated by different superscript letters (m-p) are significantly different (DMRT at 5% level)

Dose (mg/l)	% mortality of *Branchiura sowerbyi* at different times of exposure (h)			
	24	48	72	96
00	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$
4.5	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$	$10^{bn} \pm 0.43$	$10^{bn} \pm 0.43$
6.0	$00^{am} \pm 0.00$	$00^{am} \pm 0.43$	$20^{cn} \pm 0.50$	$30^{co} \pm 0.43$
7.5	$10^{bm} \pm 0.43$	$10^{bm} \pm 0.43$	$40^{dn} \pm 0.43$	$50^{do} \pm 0.00$
9.0	$20^{cm} \pm 0.43$	$30^{cn} \pm 0.43$	$50^{eo} \pm 0.00$	$70^{ep} \pm 0.43$
10.5	$30^{dm} \pm 0.00$	$50^{dn} \pm 0.43$	$70^{fo} \pm 0.43$	$90^{fp} \pm 0.43$
12.0	$40^{em} \pm 0.00$	$70^{en} \pm 0.50$	$80^{go} \pm 0.43$	$100^{gp} \pm 0.00$
13.5	$60^{fm} \pm 0.50$	$80^{fn} \pm 0.43$	$100^{ho} \pm 0.50$	$100^{go} \pm 0.00$
15.0	$80^{gm} \pm 0.43$	$100^{gn} \pm 0.00$	$100^{hn} \pm 0.00$	$100^{gn} \pm 0.00$
16.5	$100^{gm} \pm 0.43$	$100^{gm} \pm 0.00$	$100^{hm} \pm 0.00$	$100^{gm} \pm 0.00$

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