

# Acute Toxicity and Behavioral Alterations of Oligochaete Worm, *Branchiura sowerbyi* Exposed to Diazinon

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## Abstract

In the present study, acute toxicity of the organophosphate pesticide, diazinon, was evaluated under experimental condition to Oligochaete worm, *Branchiura sowerbyi*. The 96 h LC<sub>50</sub> with 95% confidence limits of *B. sowerbyi* was 2.87 (2.50–3.17) mg/l. None of the unexposed control worm died during the experiment. Mortality rate between each dose and mortality rate between 24–96 h depending on time was correlated. The mortality rate also varied significantly ( $p < 0.05$ ) with the increasing concentration for the organisms at all the exposure concentrations except 6.00 mg/l, 6.50 mg/l and 7.00 mg/l ( $p > 0.05$ ) at all the exposure times. The relationship between mortality rate and exposure times was found to be significant ( $p < 0.01$ ). The effect of toxicant on the behavioral changes of the Oligochaete worm was directly proportional to the increasing concentration of diazinon.

**Keywords:** Acute toxicity, diazinon, *Branchiura sowerbyi*, 96 h LC<sub>50</sub>, behavior

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## INTRODUCTION

The pesticides are used to eradicate different pest populations in agricultural fields for increasing food production [1]. When a pesticide is used in agricultural field, it affects its target pest population only by 0.1%, but the rest of the insecticide contaminates the natural ecosystem [2, 3]. The accumulation and persistence of pesticides in the aquatic ecosystem creates a threat to aquatic biodiversity including human beings [4]. Various investigations have revealed that pesticides are responsible for reproductive dysfunction, endocrine disruption, developmental disabilities and respiratory diseases on different nontarget organisms [5]. Diazinon is an organophosphate group of pesticide which is used as an insecticide to protect crops from a wide range of hymenopteran and hemipteran insects [6–8]. The toxic effects of diazinon is due to blocking of acetyl cholinesterase (AChE) activity that causes deleterious effects on nontarget aquatic animals near to the agricultural fields [6]. Acute toxicity of a pesticide refers to its ability to cause mortality to an animal from a single exposure of short duration [9]. Literatures available on the toxicity of diazinon on *Branchiura sowerbyi* are scanty. The earlier

studies on the toxicity of diazinon are mostly restricted to fish.

The purpose of the present investigation was to determine the sensitivity of different freshwater organisms belonging to diverse niches to find out alternative test species for ecotoxicological studies in the aquatic ecosystem, to provide further diazinon toxicity data for use in ecological risk assessment and to determine the safe disposal level of diazinon. Taking these into consideration, an attempt was made to assess the acute toxicity and behavioral alterations of *B. sowerbyi* exposed to diazinon.

## MATERIALS AND METHODS

*Branchiura sowerbyi* (Class: Oligochaeta; Family: Tubificidae), a benthic Oligochaete worm, is used as the test organism in the bioassay. The test organisms were collected from local unpolluted sources and were allowed to acclimate gradually to the test water for a minimum of 48 h. Analytical grade Diazinon (IUPAC name: *O,O*-Diethyl *O*-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate) belonging to the organophosphate class of insecticide (molecular weight 304.34 g·mol<sup>-1</sup>; with purity of 60% and dissolved in 40% acetone) was used to prepare test solutions of

diazinon. The static replacement bioassay test with worm was conducted in 500 ml glass beakers each containing 300 ml of water for the determination of acute toxicity of the test chemical following the methods of earlier workers [10–12]. A set of four beakers were exposed to a single concentration of diazinon to make four replicates per concentration. Each set of experiment was accompanied by four replicates of control. Test animals were not fed 24 h before and during the bioassays. Tap water stored in the glass aquaria (temperature  $24\pm 0.34$  °C, pH  $8.00\pm 0.31$ , free  $\text{CO}_2$   $10.5\pm 0.44$  mg/l, DO  $8.6\pm 0.34$  mg/l, alkalinity  $172\pm 2.11$  mg/l as  $\text{CaCO}_3$ , hardness  $123\pm 5.6$  mg/l as  $\text{CaCO}_3$ ) was used as a diluent medium during the experiment. The bioassays and the limnological tests were done following the methods outlined in APHA (2012). Prior to the introduction of worms in the aquaria, diazinon was added and uniform mixing of diazinon was ensured by stirring with the help of a magnetic stirrer. Two control sets were also run containing 0.6 mg/l of acetone that was used for the dilution of diazinon. The dose range causing mortality to the test organisms was determined from initial rough range finding tests (data not shown). The selected test concentrations of diazinon finally used for the determination of 96 h  $\text{LC}_{50}$  to the test organisms were 0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 mg/l. In each replicate five worms (mean length  $18\pm 4$  mm; mean weight  $1.89\pm 1.04$  mg) were used. The number of dead organisms were counted at every 24 h of experiment and removed from the

test solution to avoid organic decomposition causing depletion of dissolved oxygen. Ten percent of the test solution was replaced every 24 h by unchlorinated stock tap water and the desired quantity of diazinon was added to water to ensure a constant concentration of the toxicant in solution and also to avoid other abiotic factors interfering in the performance of the animals [13].

The rate of mortality at different concentrations and at different times of exposure (24 h, 48 h, 72 h, 96 h) was calculated using the computer software R version 2.14.0 [14, 15] and probit analysis by Finney [16] for determination of 96 h  $\text{LC}_{50}$  value with 95% confidence limit of diazinon to the Oligochaete worm. The relation between mortality rate with different doses and exposure time was determined using correlation analysis [15, 17]. The ethological changes such as restlessness, mucus secretion and wrinkling effect were also recorded [11, 18].

## RESULTS AND DISCUSSION

No organism died during the acclimatization period. The lethal concentrations of diazinon to the worm, *B. sowerbyi* are summarized in Table 1.

The present 96 h  $\text{LC}_{50}$  value of diazinon to *B. sowerbyi* was 2.87 mg/l which is slightly lower than the results of earlier workers (96 h  $\text{LC}_{50}$  value 3.16 mg/l) [19]. The differences in our findings may be associated with external factors such as temperature, water quality parameters and pH [20–23].

**Table 1:** Lethal Concentrations ( $\text{LC}_5$ ,  $\text{LC}_{10}$ ,  $\text{LC}_{50}$ ,  $\text{LC}_{90}$ ,  $\text{LC}_{95}$ ) along with 95% Confidence Limits of Diazinon to *Branchiura sowerbyi* at Different Hours of Exposure (24 h, 48 h, 72 h, 96 h).

Lethal concentrations	Concentration (mg/l)			
	24 h	48 h	72 h	96 h
$\text{LC}_5$	2.48 (2.01–2.83)	1.99 (1.56–2.33)	1.69 (1.28–2.02)	1.37 (0.96–1.70)
$\text{LC}_{10}$	2.82 (2.38–3.16)	2.30 (1.88–2.64)	1.97 (1.56–2.30)	1.61 (1.19–1.94)
$\text{LC}_{50}$	4.50 (4.18–4.84)	3.85 (3.52–4.16)	3.37 (3.04–3.66)	2.87 (2.50–3.17)
$\text{LC}_{90}$	7.17 (6.43–8.44)	6.44 (5.78–7.55)	5.75 (5.17–6.71)	5.08 (4.56–5.96)
$\text{LC}_{95}$	8.18 (7.18–9.99)	7.45 (6.54–9.07)	6.69 (5.89–8.14)	5.98 (5.24–7.35)

**Table 2:** Correlation (*r*) of Exposure Times (24 h, 48 h, 72 h and 96 h) with Mortality Rate for *Branchiura sowerbyi* to Diazinon Concentration. \*Insignificant.

Concentration	r	SE	p-value
2.00	0.948	0.299	p<0.05
2.50	0.894	0.282	p<0.05
3.00	0.944	0.298	p<0.05
3.50	0.948	0.299	p<0.05
4.00	0.944	0.298	p<0.05
4.50	0.949	0.299	p<0.05
5.00	0.949	0.299	p<0.05
5.50	0.894	0.282	p<0.05
6.00	0.774	0.244	p>0.05*
6.50	0.774	0.244	p>0.05*
7.00	0.774	0.244	p>0.05*

**Table 3:** Correlation (*r*) of Diazinon Concentration with Mortality Rate for *Branchiura sowerbyi*. \*\*Significant at 1% Level.

Exposure time (h)	r	SE	p-value
24	0.977	0.488	p<0.01**
48	0.973	0.486	p<0.01**
72	0.964	0.482	p<0.01**
96	0.938	0.469	p<0.01**

**Table 4:** Impact of Diazinon on the Behavioral Responses of *Branchiura sowerbyi* (M: Restlessness; MS: Mucus Secretion; WE: Wrinkling Effect; -: None; +: Mild; ++: Moderate; +++: Strong; X: Not Recorded due to Death) at Various Concentrations During Different Hours of Exposure.

Dose (mg/l)	24 h			48 h			72 h			96 h		
	M	MS	WE	M	MS	WE	M	MS	WE	M	MS	WE
0.00	-	-	-	-	-	-	-	-	+	-	-	-
2.00	-	-	+	-	-	+	-	-	+	-	-	-
2.50	+	+	+	+	+	+	+	+	++	+	++	+
3.00	+	+	++	++	+	++	++	+	++	++	++	++
3.50	+	+	++	++	+	++	++	+	++	++	++	++
4.00	+	++	++	++	++	++	++	++	++	++	++	++
4.50	++	++	++	+++	++	+++	+++	++	+++	+++	++	+++
5.00	++	++	++	+++	++	+++	+++	++	+++	+++	+++	+++
5.50	++	++	++	+++	++	+++	+++	++	+++	+++	++	+++
6.00	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
6.50	++	++	++	+++	+++	+++	+++	+++	+++	+++	++	+++
7.00	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++

The behavioral changes observed in the organisms exposed to diazinon are summarized in Table 4.

Initially *B. sowerbyi* showed faster movement at all the exposures but a gradual decrease in movement at all exposures was observed with increasing concentration of diazinon. This may

be due to toxic action in the body which was acute at 96 h in higher concentration [24]. With the increase of concentration mucus secretion and the wrinkling effect gradually increased for all the different hours of exposure (Table 4).

Similar observation was also recorded at higher concentration of methanol to *B. sowerbyi* [25].

## CONCLUSION

The toxicity experiment highlights the toxicity of diazinon to *B. sowerbyi* during their acute exposure. The findings on the present lethal concentration (LC) values indicate that diazinon is highly toxic to aquatic organisms (Table 1). The LC<sub>50</sub> values of the present study may provide useful data to set up national and local water quality criteria (WQC) for diazinon. The observation on the ethological responses of the organism in the present experiment may be an indicative parameter for assessing the toxicity of diazinon in the ecosystem. The data obtained from the experiment may help in ecological risk assessment and ascertain the safe levels of diazinon before being discharged to various water bodies. So, it is important to ensure proper action plan to prevent possible contamination of aquatic ecosystem when diazinon is used for agricultural purpose near freshwater bodies.

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