Journal of Aquatic Biology & Fisheries | 2020 | **8** | pp. 37-40
ISSN 2321–340X

Captan-Induced Toxicity and Behavioural Alterations on Oligochaete Worm, *Branchiura sowerbyi*

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Abstract

Toxicity of fungicide Captan to a benthic worm, *Branchiura sowerbyi* (Annelida: Oligochaeta: Archioligochaeta: Tubificidae) along with their behavioural alterations was studied in the present investigation. The 24, 48, 72 and 96 h LC50 values of Captan to *B. sowerbyi* were 10.19, 9.51, 8.97 and 8.63 mg/l respectively. The rate of mortality of *B. sowerbyi* also varied significantly ($p<0.05$) with the increasing concentration for the organisms at all the exposure concentrations except 7.00, 10.00, 10.50, 11.00, 11.50, 12.00, 12.50 and 13.00 mg/l ($p>0.05$) at all the exposure times. The relationship between the rate of mortality and exposure times (24, 48 and 72 and 96h) was found to be significant ($p<0.01$). A decrease in movement and clumping tendency along with excess mucous secretion were recorded in *B. sowerbyi* at higher concentrations.

Keywords: Acute toxicity, Captan, *Branchiura sowerbyi*, 96 h LC_{50}, Behaviour

1. Introduction

Pesticides are toxic substances released into the environment in massive amounts with the potential to cause adverse effects on human as well as wildlife populations (Galloway *et al*., 2003). Pesticides affect its target pest population only by 0.1%, but the rest of the insecticide contaminates the natural ecosystem (Hart *et al*., 2002; Mahboob *et al*., 2011). The accumulation and persistence of pesticides in the aquatic ecosystem cause a threat to aquatic biodiversity (Faruk, 2008). The non-systemic fungicide Captan which was first registered in Canada in 1953, is used to control disease in vegetables, fruits and tobacco (Saha *et al*., 2018). It has a broad industrial application for control of mould in paints, lacquers and wallpaper pastes (AAFC, 1997). Captan reacts with sulphydryl groups, and this is the principal mode of action in fungal cells (Boran *et al*., 2012). It inhibits the process of respiration and metabolism of the fungus through a thiol reactant (Barreda *et al*., 2006). Captan is responsible for the decrease of fungal spore germination, oxygen uptake and growth. Captan also has cytotoxic effects on *Drosophila melanogaster* (Nazir et al., 2003). It is highly toxic to fish, other aquatic fauna and the pollutant enters the natural water bodies through agricultural run-off, municipal sewage and industrial discharges (Saha *et al*., 2018). Literature available on the toxicity of Captan on fish is scanty. No experimental work has been conducted on the toxicity of captan on benthic oligochaete worm, *Branchiura sowerbyi*.

The purpose of this study was to assess 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 toxicity data of Captan for use in ecological risk assessment and to measure the safe disposal level of Captan to the ecosystem. Taking these into consideration, we tried to determine the acute toxicity and behavioural alterations of *Branchiura sowerbyi* exposed to Captan.

2. Materials and Methods

The benthic Oligochaete worm, *Branchiura sowerbyi* (Class: Oligochaeta; Family: Tubificidae) is used as the test animal in the present investigation. The test organisms were collected from unpolluted local sources and were allowed to acclimate to the test water for 72 h. Analytical grade Captan (3a*R*,7a*S*)-2-[(trichloromethyl) sulfanyl]-3a,4,7,7a-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione) $C_9H_8C_{13}NO_2S$, belonging to the phthalimide class of fungicides (molecular weight 300.59 g/mol; Jardine Distribution Inc.) was used to prepare test solution.

The static replacement bioassay test with worm was done in 500 ml glass beakers each containing 400 ml of water for the determination of acute toxicity of the test chemical following the methods of earlier workers (APHA, 2012; Mukherjee *et al*., 2014; Saha *et al*., 2014; Sarkar *et al*., 2018). A set of four beakers were exposed to a single concentration of Captan to make four replicates per dose. Each set of experiment was accompanied by four replicates of control. Test organisms were not fed 24 h before and during the experiment. Tap water stored in the glass aquaria (temperature 24 ± 0.34 °C, pH 8.00 ± 0.31, free CO_2 10.3 ± 0.43 mg/l, DO 8.5 ± 0.30 mg/l, alkalinity 169 ± 2.01 mg/l as $CaCO_3$, hardness 118 ± 4.8 mg/l as $CaCO_3$) was used as a diluent medium for the experiment. The bioassays and the limnological tests were conducted

Received on: 26-12-2019; Revised on: 06-09-2020; Accepted on: 13-10-2020

following the methods of APHA. The required quantity of Captan was weighed for the different test concentrations and then added directly to the test medium.

The dose range causing mortality to the test animals was determined from the initial range finding tests. The selected concentrations of Captan finally used in the experiment for the determination of 96 h LC_{50} to *B. sowerbyi* were 0, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5 and 13.0 mg/l. In each replicate five organisms (mean length 16 ± 3 mm; mean weight 1.82 ± 0.84 mg) was used. The number of dead worms was counted at every 24 h of experiment and removed from the test solution to avoid decomposition, causing the lowering of dissolved oxygen. Ten per cent of the test solution was changed every 24 h by unchlorinated stock tap water, and the required quantity of Captan was added to water to ensure a right concentration of the pesticide in solution medium and also to avoid the other factors interfering in the behaviour of the animals (Badanthadka *et al*., 2005).

The rate of mortality at different concentrations of Captan and at various times of exposures (24, 48, 72, 96 h) were calculated using the software R version 2.14.0 and the probit analysis for calculation of 96 h LC_{50} value with 95% confidence limit to the worm (R Development Core Team, 2012; US EPA, 1999; Finney, 1971). The relation between the rate of mortality with different doses and exposure time was determined using the correlation analysis (Gomez et al., 1984). The values of percentage mortality of worm were subjected to analysis of variance (ANOVA) with the help of R software followed by Duncan's Multiple Range Test (DMRT) to calculate the significant variation among the mean values at different concentrations of Captan at various times of exposure (24, 48, 72 and 96h) (R Development Core Team, 2012). The behavioural alterations like Clumping Tendency, decrease in movement, and mucus secretion were also recorded (Rand, 1985; Mukherjee *et al*., 2012).

3. Results and Discussion

The 24, 48, 72 and 96 h LC_{50} values of Captan to *Branchiura sowerbyi* were 10.19, 9.51, 8.97 and 8.63 mg/l (Table 1). The mortality rate of *B. sowerbyi* varied significantly ($p<0.05$) with the increasing concentration for the organisms at all the exposure concentrations except 7.00, 10.00, 10.50, 11.00, 11.50, 12.00, 12.50, 13.00 mg/l ($p>0.05$) at all the exposure times. The relationship between the rate of mortality and exposure times (24, 48 and 72 and 96h) was found significant ($p<0.01$) (Table 2 and 3). No organism died during the acclimatization period. The lethal concentrations of Captan to the worm, *B. sowerbyi* are summarized in Table 1.

Table 1. Lethal concentrations (LC_5, LC_{10}, LC_{50}, LC_{90}, LC_{95}) with 95% confidence limits of Captan to *Branchiura sowerbyi* at various hours of exposure (24, 48, 72, 96h).

Lethal Concentrations	Conc. (mg/l) 24 h	48 h	72 h	96 h
LC_5	7.23	6.73	5.74	5.66
	(6.51 – 7.75)	(6.01-7.25)	(4.78-6.43)	(4.74 – 6.32)
LC_{10}	7.8	7.26	6.34	6.21
	(7.16 – 8.26)	(6.62- 7.74)	(5.45-6.96)	(5.36-6.82)
LC_{50}	10.19	9.52	8.97	8.63
	(9.82 – 10.59)	(9.14 – 9.88)	(8.48-9.39)	(8.15-9.04)
LC_{90}	13.31	12.46	12.69	11.98
	(12.53 – 14.55)	(11.79-13.50)	(11.83-14.16)	(11.26-13.18)
LC_{95}	14.36	13.45	14.01	13.15
	(13.36-16.01)	(12.58-14.85)	(12.84-16.12)	(12.17-14.86)

Table 2. Correlation (r) of different exposure times (24, 48, 72 and 96 h) with the rate of mortality for *Branchiura sowerbyi* to different concentration of Captan.

Concentration	R	SE	p-value
7	0.774	0.214	p>0.05
7.5	0.948	0.262	p<0.05*
8	0.894	0.247	p<0.05*
8.5	0.894	0.247	p<0.05*
9	0.894	0.247	p<0.05*
9.5	0.894	0.247	p<0.05*
10	0.774	0.214	p>0.05
10.5	0.774	0.214	p>0.05
11	0.774	0.214	p>0.05
11.5	0.774	0.214	p>0.05
12	0.774	0.214	p>0.05
12.5	0.774	0.214	p>0.05
13	0.774	0.214	p>0.05

(*Significant at 5% level)

Table 3. Correlation (r) of Captan concentration with the rate of mortality for *Branchiura sowerbyi*

Exposure time (h)	R	SE	p-value
24	0.98	0.49	p<0.01**
48	0.968	0.484	p<0.01**
72	0.97	0.485	p<0.01**
96	0.972	0.486	p<0.01**

(**Significant at 1% level)

Table 4. Mean values (±SD) of % mortality of *Branchiura sowerbyi* exposed to various concentrations of Captan at different times of exposure (24, 48, 72 and 96h). Mean values of columns indicated by different subscript letters (a-c) and mean values of rows indicated by different subscript letters (m-r) are significantly different (DMRT at 5% level)

Dose	Mean values (±SD) of % mortality of *Branchiura sowerbyi* at different time of exposure (24h, 48h, 72h, 96h)			
Mg/L	**24h**	**48h**	**72h**	**96h**
7	0^{am}±0.00	20^{bn}±0.12	20^{bn}±0.00	20^{cn}±0.10
7.5	20^{an}±0.11	20^{an}±0.00	20^{bp}±0.10	40^{co}±0.10
8	20^{an}±0.11	20^{abo}±0.00	40^{abp}±0.10	40^{bo}±0.10
8.5	20^{an}±0.10	20^{abo}±0.10	40^{abp}±0.10	40^{bo}±0.08
9	40^{ao}±0.15	40^{ap}±0.09	60^{bp}±0.08	60^{cp}±0.08
9.5	40^{ao}±0.09	40^{ap}±0.05	60^{bp}±0.13	60^{cp}±0.09
10	40^{ao}±0.12	60^{bp}±0.09	60^{cq}±0.10	60^{cp}±0.08
10.5	60^{ap}±0.16	80^{aq}±0.05	80^{br}±0.16	80^{bq}±0.10
11	60^{ap}±0.16	80^{aq}±0.10	80^{br}±0.09	80^{bq}±0.16
11.5	80^{aq}±0.12	80^{ar}±0.16	80^{ar}±0.09	90^{aq}±0.05
12	80^{aq}±0.09	90^{ar}±0.09	90^{ar}±0.10	100^{aq}±0.00
12.5	80^{aq}±0.10	90^{ar}±0.05	100^{ar}±0.00	100^{aq}±0.00
13	100^{aq}±0.08	100^{ar}±0.00	100^{ar}±0.00	100^{aq}±0.00

Table 5. Impact of Captan on the behavioural responses of *Branchiura sowerbyi* (M: movement; MS: mucus secretion; CT: Clumping Tendency; -: absent; +: mild; ++: moderate; +++: strong; X: not recorded for death) at different concentrations during various hours of exposure.

Dose	**24 h**			**48 h**			**72 h**			**96 h**		
(mg/l)	**M**	**MS**	**CT**	**M**	**MS**	**CT**	**M**	**MS**	**CT**	**M**	**MS**	**CT**
0	++	-	+++	++	-	+++	++	-	+++	++	-	+++
7	++	-	+++	++	-	+++	++	-	+++	++	-	++
7.5	++	-	+++	++	-	+++	++	-	++	+	-	++
8	++	-	+++	++	-	+++	++	-	++	+	+	+
8.5	++	-	+++	++	-	++	+	+	++	+	+	+
9	++	-	+++	+	-	++	+	+	+	+	+	+
9.5	+	-	++	+	+	+	+	+	+	+	++	-
10	+	-	++	+	+	+	+	+	+	-	++	-
10.5	+	+	++	+	+	+	+	++	+	-	++	-
11	+	+	++	+	+	+	-	++	-	-	++	-
11.5	+	+	+	-	+	-	-	++	-	-	++	-
12	+	+	+	-	++	-	-	++	-	X	X	X
12.5	-	++	+	-	++	-	X	X	X	X	X	X
13	X	X	X	X	X	X	X	X	X	X	X	X

The behavioural changes observed in the organisms exposed to Captan are summarized in Table 5.

In the present study, abnormal behaviour like erratic movement, excess mucous secretion and clumping tendency observed were may be for the enzymatic as well as an ionic alteration in *B. sowerbyi* (Larsson *et al*., 1981). *B. sowerbyi* showed faster movement initially at all the exposures. Still, decrease in movement was observed with increasing concentration of Captan. This may be due to the toxic action of Captan which was acute at 96 h in higher concentration (Mukherjee *et al*., 2012). The clumping tendency of *B. sowerbyi* inversely varies with increasing concentration of Captan and exposure times. The excessive mucous secretion was observed in worm at higher concentrations of Captan at 72 and 96h of exposure. Probably, this excessive mucous secretion was an indication of avoidance reaction from the pesticide (Sarkar *et al*., 2018). The toxic effects of Captan to worm may be for the formation of a mucous-toxicant compound which covers the body of the worm and inhibits the exchange of O_2 and CO_2 (Whitley, 1967).

4. Conclusion

The findings on the present lethal concentration (LC) values indicate that Captan is highly toxic to aquatic organisms. The perturbation of community function and in the food chain is quite evident as the worm is sensitive to Captan at low concentration. The maximum permissible limit of captan for discharge into aquatic bodies may be established based on the LC50 values of the present experiment. Besides, a complete picture on the toxicity of Captan can be derived based on the study of toxicity imparted by its various metabolites.

Acknowledgements

We are thankful to the Head of the Department of Zoology, Burdwan University, for providing infrastructural facilities for the study. The authors are also very grateful to Arnab Chatterjee for his support.

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