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# Acute toxicity and ethological responses of oligochaete worm *Tubifex tubifex* (Muller) exposed to a cationic surfactant Cetylpyridinium chloride

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*Abstract*—Cetylpyridinium chloride is a quaternary cationic surfactant which exhibit broad spectrum antimicrobial activity. The present study was conducted to evaluate the acute toxicity of Cetylpyridinium chloride to a benthic oligochaete worm *Tubifex tubifex* in terms of  $LC_{50}$  and behavioral responses. All experiments were conducted, in a static bioassay system, using increasing concentrations of Cetylpyridinium chloride for 96 h duration. The  $LC_{50}$  values associated with 95% lower and upper confidence limits for the surfactant were determined statistically using Finney's Probit Analysis Method developed by EPA and were found to be 0.330 (0.305-0.357) ppm, 0.305 (0.280-0.332) ppm, 0.225 (0.204-0.248) ppm and 0.213 (0.190-0.240) ppm at 24, 48, 72 and 96 hours exposure respectively. The results of regression analysis indicated that the mortality rate varied significantly (p<0.05) with the increasing concentrations of surfactant. In addition, the aquatic worm exhibited abnormal behavioral responses like hyperactivity, decreased clumping tendency, increased mucous secretion and wrinkling effect on increasing concentrations of toxicant and period of exposure. Thus, these findings can be used as a potential tool for creating awareness among people to limit the misuse of household products containing surfactants.

Keywords— Acute toxicity, Cetylpyridinium chloride, Tubifex tubifex, behavioral response

# I. INTRODUCTION

Water is the most important component for the survivability of life. It is an essential constituent of all flora and fauna. But exorbitant discharge of household, domestic effluents, and detergents into the water bodies contaminate the water causing the death of non-target aquatic organisms. Surfactants, one of the crucial components of household products, impose an adverse impact on the water bodies resulting in enhanced mortality of aquatic fauna [1]. One such surfactant extremely commercialized cationic is Cetylpyridinium chloride (CPC), a quaternary cationic ammonium compound, specifically utilized in mouthwash and toothpaste for obviation of tooth plaques and periodontal disease [2], [3], [4]. Additionally, it also removes reactive dyes, phenols, and different organic solutes from waste products or treated effluents [5], [6], [7].

Several studies were conducted relating to the oral intoxication of CPC to rats and mice [8],[9]. But studies regarding its hazardous impact to benthic aquatic organisms are scanty. In the present study, the benthic oligochaete worm, *Tubifex tubifex* was selected as a test organism because it is an important benthic bio indicator species, cosmopolitan in distribution, possess a high tolerance to a

diverse array of environmental variables and additionally serves as a healthy food source of fishes specifically fresh water ornamental ones [10],[11],[12],[13],[14].

Thus the present investigation was conducted to determine the acute toxicity of CPC in terms of  $LC_{50}$  and behavioral responses as an indicator of chemical stress to *Tubifex tubifex*.

The paper is organized as follows, Section I contains the Introduction of the toxicant, CPC and its effect on aquatic ecosystem. Section II contains some related works regarding the toxicity of CPC. Section III contains the methodology employed to pursue the present study. Section IV describes the results and discussions associated with tables and graphical representations and Section V concludes the research work with future scopes.

# II. RELATED WORKS

Rosen et al, (1965) "Dimethyl sulfoxide (DMSO) as a solvent in acute toxicity determinations" – evaluated the acute toxcity of CPC on both rats and mice in the presence of distilled water and dimethyl sulfoxide (DMSO) [8]. Nelson et al, (1946)"The toxicity of myristyl-gammapicolinium chloride" – determined the LD<sub>50</sub> value of CPC in rats and the values are 250 mg./Kg. subcutaneously, 6 mg./Kg. intraperitoneally, 30 mg./Kg. intravenously, and 200 mg./Kg. Orally [9].

M.H. Li, (2012) "Survival, mobility, and membrane-bound enzyme activities of freshwater planarian, *Dugesia japonica*, exposed to synthetic and natural surfactants" – studied the acute toxicity and locomotary changes of fresh water planarian *Dugesia japonica* upon exposure to CPC [15].

# III. METHODOLOGY

#### Collection of the test organism

The bio indicator species, *Tubifex tubifex* were collected from the local market of Burdwan, West Bengal, India and acclimatized in the laboratory for a period of 24 h in unclorinated pollution free water (temperature  $26.5 \pm 0.2$  °C, pH 7.4 ± 0.4, free CO<sub>2</sub> 16.4 ± 0.7 mg/l, dissolved oxygen 6.9 ± 0.2 mg/l, total alkalinity 187 ± 7.3 mg/l as CaCO<sub>3</sub>, hardness 118 ± 4.9 mg/l as CaCO<sub>3</sub>). Then healthy organisms with a mean length of 11.6 ± 0.4 mm were transferred to the experimental system.

#### Test chemical used

Technical grade of CPC was procured from SRL and used as a test chemical. Its stock solution (1%) and dilutions were made following the APHA method (2012) [16].

#### Acute toxicity bioassay

To determine the LC<sub>50</sub> value of CPC, 96 hours static renewal acute toxicity test was conducted in a controlled laboratory condition by exposing Tubifex tubifex (n=10) to different concentrations of CPC. Each experiment was conducted in triplicates. Initially a range finding test was piloted to find out the range of the concentrations where mortality occurs. Thereafter a definitive test was conducted by exposing the worms to different concentrations of surfactant i.e. 0.1, 0.125, 0.15, 0.175, 0.2, 0.225, 0.25, 0.275, 0.3, 0.325, 0.35, 0.375, 0.4, 0.425 mg/l along with a control. Based on the toxicant dose and percent mortality at 24, 48, 72 ad 96 hrs, the  $LC_{50}$ values along with 95 % confidence limits were determined by using Finney's probit analysis method [17]. The behavioural responses of the worm were observed and recorded at different exposure periods by employing semi quantitative scoring method [18]. The toxicological end points like LOEC (Lowest Observed Effect Concentration), NOEC (No Observed Effect Concentration) at 96h were determined based on the acute toxicity data. MATC (Maximum Acceptable Toxicant Concentration) was calculated by multiplying 96h LC<sub>50</sub> value with Application Factor 0.1 [19].

#### Determination of Safe permissible limit of the toxicant

The safe level of CPC was calculated by using Application Factors based on Edwards and Brown [20], Burdick [21], Sprague [22] Water Quality Criteria (CWQC) [23], National Academy of Science/National Academy of Engineering (NAS/NAE) [24], International joint commission [25], and Canadian council of Resources and Environmental Ministry (CCREM) (IJC) [26].

#### **Statistical Analysis**

The statistical analysis was carried out by employing Graphpad prism 7.0. The linear regression curves were drawn by employing MS Excel 2013.

#### IV. RESULTS AND DISCUSSION

#### Acute toxicity and determination of 96 hrs. LC<sub>50</sub>

No mortality was observed in controlworms as well as worms treated with 0.1 mg/lit of toxicant throughout 96 h. However, with the further increment of the concentrations of the toxicant and the exposure period, the mortality rate increased (Table 1). This observation designated that mortality is dose and time dependent. The  $LC_{10}$  to  $LC_{90}$ values of CPC observed in the 24, 48, 72 and 96h exposure period were represented in Table 2. The 24, 48, 72 and 96h LC<sub>50</sub> values, 95% confidence limits, and correlation coefficients of CPC at different exposure periods for Tubifex tubifex are summarized in Table 3 which indicates an existence of a strong positive correlation between the percentage mortality and toxicant concentration. The regression plots of probit mortality against Log<sub>10</sub> concentrations of CPC for 24, 48, 72 and 96 h. have been depicted in Figure 1. The NOEC, LOEC and MATC values at 96h were determined and compared with the subsequent  $LC_{50}$ value which is depicted in Figure 2. The calculated values of LOEC, NOEC and MATC are 0.125, 0.1 mg/l and 0.0213 mg/l respectively.

# Behavioural changes in *Tubifex tubifex* due to acute toxicity

Upon addition of CPC at different exposure periods, the *Tubifex tubifex* exhibited dose and duration dependent irregular behavioural responses which are represented in Table 4. The control worms exhibited normal behavioural attributes throughout the exposure period. But a decline in the clumping propensity and increment in hyperactivity, wrinkling effect, and mucous secretion of the exposed worms were observed with increasing concentrations of toxicant and exposure times.

#### Safe concentrations

The safe permissible limits of CPC calculated for the oligochaete worm *Tubifex tubifex* are represented in Table 5 and is found to be within the range of 0.0000213 - 0.01065 ppm.

#### Discussion

In the present investigation, *Tubifex tubifex* exhibited variations in mortality rate with the incrementing surfactant concentrations and exposure period. In the present study, the

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96h LC<sub>50</sub> value of CPC to *Tubifex tubifex* is estimated to be 0.213 mg/l which is much higher than the other aquatic animals as reported to be 0.04 mg/l in case of fresh water planarian (*Dugesia japonica*) [15] and 0.01 mg/l in case of common carp (*Cyprinus carpio*) according to Reagentworld Material Safety Data Sheet.

According to the value of 96h  $LC_{50}$  of CPC, it is regarded as highly toxic to aquatic organisms. However its toxicity may vary depending on temperature, pH, alkalinity and hardness of the water.

The behavioural changes are specifically related to intricate physiological responses and have often been utilized as a sensitive stress designator [27]. In our present investigation, the vicissitudes of clumping propensity, hyperactive movement, incremented wrinkling effect and enhanced mucous discharge of *Tubifex tubifex* upon exposure to CPC suggest that the tubificid worms have been subjected to chemical stress when exposed to surfactant and can be considered as a marker of aquatic contamination. Parallel trends in behavioural changes were observed when similar type of aquatic oligochaete worms *Branchiura sowerbyi* were exposed to pesticide like alpha-cypermethrin and heavy metal like Cadmium [28],[29].

The 96h LC<sub>50</sub> of CPC to *Tubifex tubifex* is also useful in determining the range of the safe permissible limit of the toxicant (0.0000213 – 0.01065 ppm). If the amount of the surfactant entering the aquatic water body exceeds the range of its safe permissible limit, it might impose detrimental effect on the survivability of this economically important bio-indicator species as well as fish population.

# V. CONCLUSION AND FUTURE SCOPE

The present investigation exhibited that the mortality rate of Tubifex tubifex upon addition of CPC is dose and duration dependent. It is exposed from the work that CPC is prodigiously toxic to aquatic bio indicator species, Tubifex tubifex based on its LC<sub>50</sub> values which were estimated to be 0.330, 0.315, 0.225 and 0.213 mg/l for 24, 48, 72 and 96 hrs. exposure period respectively. Moreover, the study also establishes the significance of behavioural parameters as a designator of chemical stress in case of benthic aquatic organisms. Thus the present findings regarding the toxicity of CPC to Tubifex tubifex may be used as a potential tool for creating awareness among people to restrict the exorbitant utilization of surfactants and preventive measures should be undertaken before its disposal to the aquatic environment to eschew any eco-toxicological hazards. However further studies are required regarding the changes in status of antioxidant enzymes in case of Tubifex tubifex upon addition of this surfactant.

#### **CONFLICT OF INTEREST**

The authors have no conflict of interest.

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CPC Conc.	CPC Conc.	Log Conc.	No of worms	24 hou	ırs	48 hours		72 ho	96 hours		
(mg/lit)	(µg/lit)	(µg/lit)	exposed	% Mortality	Probit kill						
0.1	100	2	10	0	-	0	-	0	-	0	-
0.125	125	2.10	10	0	-	0	-	0	-	10	3.72
0.15	150	2.18	10	0	-	0	-	10	3.72	20	4.16
0.175	175	2.24	10	0	-	0	-	20	4.16	30	4.48
0.2	200	2.30	10	0	-	10	3.72	40	4.75	40	4.75
0.225	225	2.35	10	10	3.72	20	3.72	50	5.00	50	5.00
0.25	250	2.40	10	10	3.72	20	4.16	60	5.25	60	5.25
0.275	275	2.44	10	20	4.16	40	4.75	70	5.52	70	5.52
0.3	300	2.48	10	40	4.75	50	5.00	80	5.84	90	6.28
0.325	325	2.51	10	50	5.00	50	5.00	90	6.28	100	-
0.35	350	2.54	10	50	5.00	70	5.52	100	-	100	-
0.375	375	2.57	10	70	5.52	80	5.84	100	-	100	-
0.4	400	2.60	10	80	5.84	100	-	100	-	100	-
0.425	425	2.63	10	100	-	100	-	100	-	100	-

Table 1: Correlation between CPC concentrations & mortality rate of *Tubifex tubifex* at different hours of exposure

Critical Level	Lethal concentration value at different exposure period (ppm)						
	24h	48h	72h	96h			
LC <sub>10</sub>	0.235	0.211	0.149	0.129			
LC <sub>20</sub>	0.264	0.239	0.172	0.153			
LC <sub>30</sub>	0.287	0.262	0.190	0.173			
LC <sub>40</sub>	0.309	0.283	0.208	0.193			
LC <sub>50</sub>	0.330	0.305	0.225	0.213			
LC <sub>60</sub>	0.353	0.328	0.244	0.236			
LC <sub>70</sub>	0.379	0.354	0.266	0.262			
LC <sub>80</sub>	0.412	0.388	0.295	0.297			
LC <sub>90</sub>	0.463	0.441	0.339	0.354			

**Table 2:** Acute toxicity of CPC to *Tubifex tubifex* at different exposure periods

 Table 3: The LC<sub>50</sub> values, 95% confidence limits, and Correlation Coefficients of CPC to *Tubifex tubifex* at different exposure periods (24, 48, 72 and 96 h)

Exposure period	LC <sub>50</sub> value ± SE (ppm)	95% confidence limit		Correlation coefficient (r)
		Lower	Upper	
24 h	$0.330 \pm 0.017$	0.305	0.357	0.979*
48 h	$0.305 \pm 0.019$	0.280	0.332	0.978*
72 h	$0.225\pm0.022$	0.204	0.248	0.994*
96 h	$0.213 \pm 0.026$	0.190	0.240	0.973*

\*Values indicate statistical significance at 0.01 levels (P < 0.01)

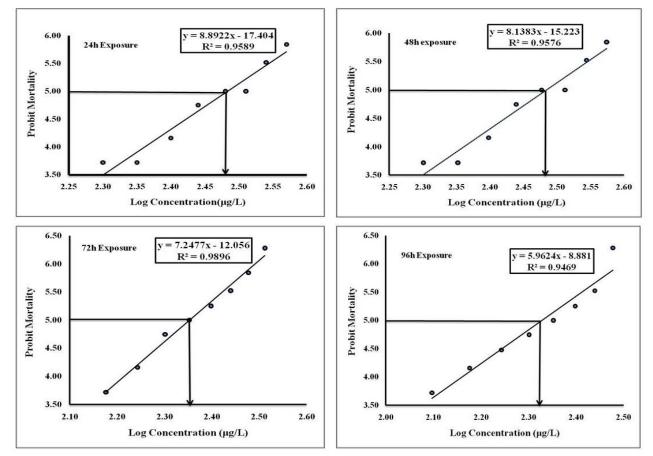


Fig 1: Regression plot between Log concentration and probit mortality of CPC to *Tubifex tubifex* at different exposure periods (p<0.05).

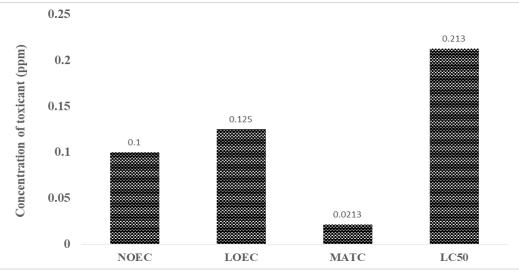


Fig 2: Comparison between NOEC, LOEC, MATC and LC<sub>50</sub> value of the toxicant at 96h exposure

**Table 4**: Impact of CPC on behavioural responses of *Tubifex tubifex (HM: Hyperactive movement; CT: clumping tendency;* 

 MS: mucus secretion; WE: wrinkling effect; -: none; +: mild; ++: moderate; +++: strong) at different concentrations in different exposure period

	Behavioral Responses of <i>Tubifex tubifex</i> upon addition of CPC													
Time of	Behavioural parameters	Dose of CPC (ppm)												
exposure (hrs.)		0.125	0.15	0.175	0.2	0.225	0.250	0.275	0.3	0.325	0.350	0.375	0.4	0.425
24	HM	-	+	+	+	++	++	++	++	++	+++	+++	+++	+++
	CT	+++	+++	+++	+++	+++	++	++	++	++	+	+	+	+
	MS	-	-	-	-	-	+	+	++	++	++	+++	+++	+++
	WE	-	-	-	-	-	-	+	+	++	++	++	+++	+++
48	HM	-	+	+	+	+	+	++	++	++	++	++	++	+++
	СТ	+++	+++	++	++	++	++	++	+	+	+	+	-	-
	MS	-	-	-	-	+	+	++	++	+++	+++	+++	+++	+++
	WE	-	-	-	-	+	+	++	++	++	++	+++	+++	+++
72	HM	-	-	-	+	+	+	+	+	+	+	++	++	++
	СТ	++	++	++	++	+	+	+	+	-	-	-	-	-
	MS	-	-	-	-	-	+	++	++	++	+++	+++	+++	+++
	WE	-	-	-	-	+	+	+	++	+++	+++	+++	+++	+++
96	HM	-	-	-	-	-	+	+	+	+	+	+	+	+
	СТ	++	++	++	++	+	+	+	+	+	+	+	+	+
	MS	-	-	-	+	+	+	+	+	++	++	++	++	++
	WE	-	-	-	+	+	+	+	++	++	++	+++	+++	+++

Table 5: Safe permissible	limit of CPC to oligochaete wor	m <i>Tubifex tubifex</i>

Surfactant	96 h LC <sub>50</sub> (ppm)	Method	Application Factor (AF)	Safe Level(mg/l)		
		Edwards and Brown (1966)	0.4	0.0852		
		Burdick (1967)	0.1	0.0213		
CPC	0.213	Sprague(1971)	0.1	0.0213		
cre	0.215	CWQC (1972)	0.01	0.00213		
		NAS/NAF (1973)	0.1-0.0001	0.0213-0.0000213		
		IJC (1977)	5% of 96h LC50	0.01065		
		CCREM (1991)	0.05	0.01065		

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