

# Sodium Dodecyl Sulphate induced acute toxicity and ethological responses to oligochaete worm *Tubifex tubifex* (Muller)

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## ARTICLE DETAILS

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## ABSTRACT

The present study was conducted to evaluate the acute toxicity in terms of 96h LC<sub>50</sub>, and behavioral responses of an anionic surfactant Sodium Dodecyl Sulphate to an oligochaete worm *Tubifex tubifex*. All experiments were conducted in a static bioassay system, using increasing concentrations of SDS for 96 h duration. The LC<sub>50</sub> values associated with 95% lower and upper confidence limits for the surfactant were determined statistically using Finney's probit analysis method and were found to be 31.370 (26.610-36.981), 18.845 (15.153-23.437), 16.325 (13.285- 20.062) and 10.940 (8.414-14.224) mg/l at 24, 48, 72 and 96 h respectively. The result of regression analysis indicated that the mortality rate varied significantly ( $p < 0.05$ ) and is positively correlated with the increasing concentrations of surfactant. Variations in behavioural responses were also recorded upon addition of different concentrations of surfactant in different exposure periods (24, 48, 72 & 96 h).

## 1. Introduction

Water is the most vital component and an essential constituent for the survivability of all flora and fauna. But contamination of water by inordinate discharge of household effluents and industrial wastes has now become a matter of great concern. Surfactants, one of the crucial components of household products, impose an adverse impact on the water bodies leading to enhanced mortality of aquatic fauna (Yuan et al., 2014). One such highly commercialized anionic surfactant is Sodium Dodecyl Sulfate (SDS) which is commonly and most widely used in household, cosmetics and laundry purposes (Chaturvedi and Kumar, 2010).

It has been reported that SDS is toxic and affects survivability of aquatic animals such as fishes, microbes like yeasts and bacteria (Chaturvedi and Kumar, 2010). It is also documented that SDS adversely affects metabolism and swimming capacity of fish, *Cyprinus carpio* (Barbieri et al., 1998). Toxic effects of SDS on the survival, metabolism, and growth of juveniles of *Centropomus parallelus* have also been reported (Rocha et al., 2007). It is also toxic to mammals in a minimal extent (Fendinger et al., 1994). But very few studies were conducted regarding the toxic effect of this surfactant to benthic oligochaete worms. In the present study, the benthic oligochaete worm, *Tubifex tubifex* was selected as a test organism because it is an important bio indicator species, possess a high tolerance to a wide range of environmental factors and additionally serves as a nutritious food source of both freshwater and ornamental fishes (Chapman, 2001, Aston, 1973, Kaster, 1980, Chong and Hashim, 2000, Tamaru, 1997).

Thus the present investigation was conducted to determine the acute toxicity of SDS in terms of LC<sub>50</sub> and behavioral responses as an indicator of chemical stress to *Tubifex tubifex*.

## 2. Methodology

### Collection of the test organism

The bio indicator species, *Tubifex tubifex* were collected from the local market of Burdwan, West Bengal, India and were acclimatized in the laboratory for a period of 24 h in unchlorinated water (Temperature  $26.4 \pm 0.4$  °C, pH  $7.5 \pm 0.3$ , free CO<sub>2</sub>  $18.8 \pm 0.4$  mg/l, dissolved oxygen  $6.1 \pm 0.8$  mg/l, total alkalinity  $184 \pm 6.8$  mg/l as CaCO<sub>3</sub>, hardness  $110 \pm 4.2$  mg/l as CaCO<sub>3</sub>). Then healthy organisms with a mean length of  $10.3 \pm 0.4$  mm were transferred to the experimental system.

### Test chemical used

Technical grade of SDS was purchased from SRL and used as a test chemical. Its stock solution (1%) and dilutions were prepared following standard protocol (APHA, 2012).

### Acute toxicity bioassay

To determine the LC<sub>50</sub> value of SDS, static renewal acute toxicity test was conducted in a controlled laboratory condition by exposing *Tubifex tubifex* (n=10) to different concentrations of SDS for a period of 96 hours. Each experiment was conducted in triplicates. Initially a rough test was conducted to extract the range of the concentrations where mortality occurs. Thereafter a definitive test was conducted by exposing the worms to different concentrations of surfactant along with a control. The mortalities were recorded at 24, 48, 72 and 96 h. Based on the toxicant dose and percent mortality at 24, 48, 72 and 96 h, the LC<sub>50</sub> values along with 95% confidence limits were determined by using Finney's probit analysis method, 1971. The behavioural responses of the worm were observed and recorded at different exposure periods (24, 48, 72 & 96 h) by using semi quantitative scoring method (Sarkar et al., 2016).

### Determination of Safe permissible limit of the toxicant

The safe level of SDS was calculated by using Application Factors based on Edwards and Brown, Burdick, Sprague, Water Quality Criteria (CWQC), International joint commission

(IJC) and Canadian council of Resources and Environmental Ministry (CCREM)

### 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. The data was subjected to two-way ANOVA associated with Post hoc multiple comparison test to determine significance differences at 5% level ( $p < 0.05$ ).

## 3. Results

### Acute toxicity and determination of 96 hrs. $LC_{50}$

No mortality was observed in control worms. However, with the further increment of the concentrations of the toxicant and the exposure period, the mortality rate increased significantly (Table 1 and Figure 1). This observation designated that mortality is dose and time dependent. The  $LC_{10}$  to  $LC_{90}$  values of SDS observed in the 24, 48, 72 and 96 h exposure period are represented in Table 2. The 24, 48, 72 and 96 h  $LC_{50}$  values, regression equations, chi square values and correlation coefficients of SDS 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  $\log_{10}$  concentrations of SDS for 24, 48, 72 and 96 h have been depicted in Figure 2.

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

With the increasing concentrations of toxicant and the progress of exposure time, *Tubifex tubifex* exhibited various erratic behaviours which are shown in Table 4. The control worms exhibited normal behaviour throughout the study. But there is a decline in clumping tendency as well as increase in mucous secretion, wrinkling effect and change in hyperactive movement with incrementing toxicant concentrations and exposure period. Mild hyperactive movement was first observed at the toxicant dose of 15 mg/L and maximum hyperactivity was noticed at higher doses (30 mg/l and above) of toxicant. But with increasing exposure periods (24, 48, 72 and 96 h), the hyperactivity gets reduced. Mucous secretion and wrinkling effect were initially noticed at the dose of 20 mg/l of toxicant and gradually gets increased upto the toxicant dose of 45 mg/l. Strong clumping tendency was observed at control as well as 5mg/l and 10 mg/l dose of toxicant but gradually gets decreased at higher doses and above the toxicant dose of 30 mg/l, no clumping tendency was observed. Moreover, clumping tendency also gets progressively decreased with incrementing exposure periods (24, 48, 72 and 96 h).

### Safe concentration range of SDS

The safe permissible limits of SDS was calculated for the oligochaete worm *Tubifex tubifex* based on the 96 h  $LC_{50}$  value and is found to be within the range of 0.01065– 4.376 mg/l (Table 5).

## 4. Discussion

In this study, the 96h  $LC_{50}$  value of SDS to *Tubifex tubifex* is 10.940 mg/l which is higher than the other aquatic animals

especially fishes. The 96 h  $LC_{50}$  values were reported to be 7.5 mg/l for *Scophthalmus maximus* (Rodríguez et al., 2002), 6.1mg/l for gilthead (*Sparus aurata*, L) (Rosety et al., 1997), 4.5 mg/l for *Fundulus heteroclitus* (Laroche et al., 1972), and 2.19 mg/l for *Mugil curema* (Gomez et al., 1984). According to our value of 96 h  $LC_{50}$  of SDS to *Tubifex tubifex*, it is regarded as moderately toxic to aquatic benthic oligochaete worms. However its toxicity may vary depending on aquatic temperature, pH, alkalinity and hardness (Chatterjee et al., 2019).

The behavioural changes are explicitly related to complex physiological responses and have often been used as an important designator of stress (Little and Finger, 1990). In our present investigation, the alterations of clumping tendency, hyperactive movement, incrementing wrinkling effect and enhanced mucous discharge of *Tubifex tubifex* upon exposure to SDS with respect to control suggest that the tubificid worms have been subjected to chemical stress when exposed to surfactant and can be considered as an indicator of aquatic contamination. Parallel trends in behavioural changes were observed in *Branchiura sowerbyi* after acute exposure to pesticide like alpha-cypermethrin and heavy metal like Cadmium (Bej et al., 2015, Dhara et al., 2015).

The range of safe permissible limit of the toxicant in our study is 0.01065– 4.376 mg/l. 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.

## 5. Conclusion

The present investigation displayed that the mortality rate of *Tubifex tubifex* upon addition of SDS is dose and duration dependent. It is revealed from the work that SDS is moderately toxic to aquatic bio indicator species, *Tubifex tubifex* based on its  $LC_{50}$  values which were estimated to be 31.370, 18.845, 16.325 and 10.940 mg/l for 24, 48, 72 and 96 h exposure period respectively. Additionally, the study also establishes the importance of behavioural parameters as an indicator of chemical and environmental stress in case of benthic aquatic organisms. Thus the present findings regarding the toxicity of SDS to *Tubifex tubifex* may be used as a potential tool for creating awareness among people to restrict the excess use of surfactants and preventive measures as well as utmost care should be undertaken before its disposal to the aquatic environment to avoid any eco-toxicological hazards.

## 6. Conflict of interest

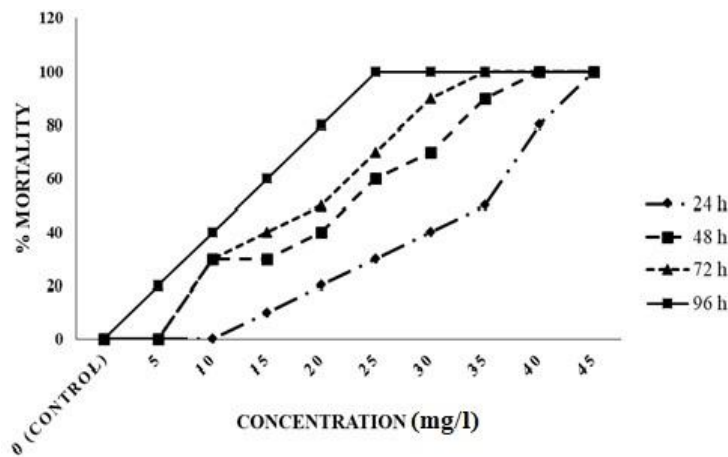
The authors have no conflict of interest.

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**Table 1:** Mean values ( $\pm$ SD) of % mortality of *Tubifex tubifex* exposed to different concentrations of SDS at different times of exposure (24, 48, 72 and 96 h). The mortality increases significantly with increasing concentration and time of exposure. 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.

Concentration (mg/l)	% Mortality			
	24h	48h	72h	96h
0 (Control)	0 $\pm$ 0 <sup>am</sup>	0 $\pm$ 0 <sup>am</sup>	0 $\pm$ 0 <sup>am</sup>	0 $\pm$ 0 <sup>am</sup>
5	0 $\pm$ 0 <sup>am</sup>	0 $\pm$ 0 <sup>am</sup>	0 $\pm$ 0 <sup>am</sup>	20 $\pm$ 0 <sup>bn</sup>
10	0 $\pm$ 0 <sup>am</sup>	30 $\pm$ 2.5 <sup>bn</sup>	30 $\pm$ 4 <sup>bn</sup>	40 $\pm$ 4 <sup>co</sup>
15	10 $\pm$ 3.5 <sup>bm</sup>	30 $\pm$ 2.5 <sup>bn</sup>	40 $\pm$ 5.5 <sup>co</sup>	60 $\pm$ 3.5 <sup>dp</sup>
20	20 $\pm$ 2 <sup>cm</sup>	40 $\pm$ 3 <sup>cn</sup>	50 $\pm$ 3.5 <sup>do</sup>	80 $\pm$ 2 <sup>ep</sup>
25	30 $\pm$ 4.5 <sup>dm</sup>	60 $\pm$ 5.5 <sup>dn</sup>	70 $\pm$ 3 <sup>eo</sup>	100 $\pm$ 0 <sup>fp</sup>
30	40 $\pm$ 4 <sup>em</sup>	70 $\pm$ 4.5 <sup>en</sup>	90 $\pm$ 2.5 <sup>fo</sup>	100 $\pm$ 0 <sup>fp</sup>
35	50 $\pm$ 2.5 <sup>fm</sup>	90 $\pm$ 5 <sup>fn</sup>	100 $\pm$ 0 <sup>go</sup>	100 $\pm$ 0 <sup>fo</sup>
40	80 $\pm$ 3 <sup>gm</sup>	100 $\pm$ 0 <sup>gn</sup>	100 $\pm$ 0 <sup>gn</sup>	100 $\pm$ 0 <sup>fn</sup>
45	100 $\pm$ 0 <sup>hm</sup>	100 $\pm$ 0 <sup>gm</sup>	100 $\pm$ 0 <sup>gm</sup>	100 $\pm$ 0 <sup>fm</sup>



**Fig 1:** Graphical representation of concentration of toxicant and mean percent mortality at different exposure period (24, 48, 72 and 96 h)

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

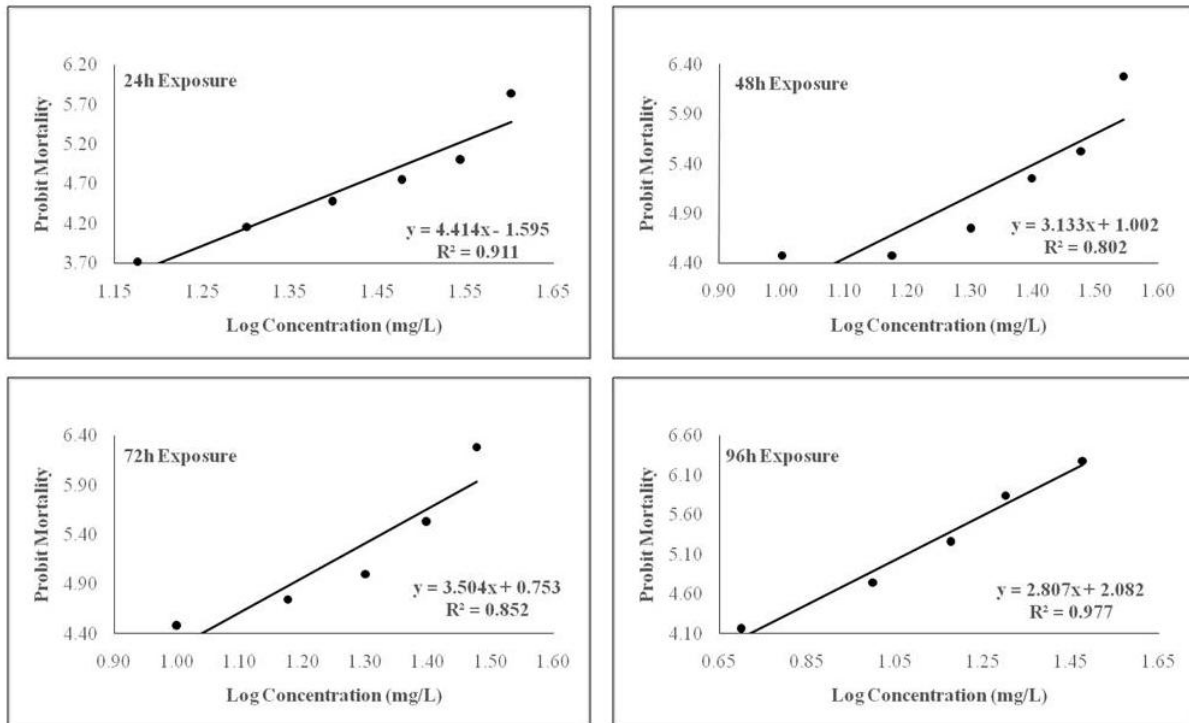
Critical Level	Lethal concentration value at different exposure period (mg/l)			
	24h	48h	72h	96h
LC <sub>10</sub>	15.884 (13.474 -18.726)	6.951 (5.589- 8.644)	6.882 (5.551- 8.383)	3.807 (2.928 - 4.949)
LC <sub>20</sub>	20.064 (17.020-23.653)	9.789 (7.871- 12.174)	9.204 (7.490- 11.311)	5.469 (4.206 - 7.111)
LC <sub>30</sub>	23.745 (20.142-27.993)	12.530 (10.075- 15.583)	11.423 (9.296- 14.038)	7.103 (5.463 - 9.235)
LC <sub>40</sub>	27.421 (23.260-32.326)	15.473 (12.441- 19.243)	13.739 (11.180- 16.884)	8.880 (6.829 - 11.545)
<b>LC<sub>50</sub></b>	<b>31.370</b> <b>(26.610- 36.981)</b>	<b>18.845</b> <b>(15.153- 23.437)</b>	<b>16.325</b> <b>(13.285- 20.062)</b>	<b>10.940</b> <b>(8.414 - 14.224)</b>
LC <sub>60</sub>	35.887 (30.441- 42.306)	22.952 (18.455- 28.545)	19.399 (15.786- 23.840)	13.479 (10.367 - 17.526)
LC <sub>70</sub>	41.442 (35.154- 48.856)	28.343 (22.790- 35.249)	23.331 (18.985- 28.672)	16.851 (12.961 - 21.910)
LC <sub>80</sub>	49.045 (41.603- 57.819)	36.280 (29.172- 45.121)	28.957 (25.563- 35.585)	21.884 (16.831 - 28.453)
LC <sub>90</sub>	61.952 (52.551- 73.034)	51.094 (41.083- 63.554)	39.070 (31.792- 48.013)	31.443 (24.183- 40.881)

**Table 3:** LC<sub>50</sub> value of SDS with Regression equations, Chi-square values and Correlation Coefficients. The positive correlation coefficients (r) indicate that percentage mortality increases significantly (P < 0.05) with increasing concentrations. The Chi-square value indicate no significant differences (P > 0.05) between observed and expected mortalities

Exposure period (h)	LC <sub>50</sub> ± SEM (mg/L)	Regression Equation	Chi Square	Correlation Coefficient
24	31.370 ± 0.036	y=4.4145x-1.5958	0.966 <sup>#</sup>	0.954 <sup>*</sup>
48	18.845 ± 0.048	y=3.1338x+ 1.002	0.884 <sup>#</sup>	0.895 <sup>*</sup>
72	16.325± 0.046	y=3.5045x + 0.7534	0.876 <sup>#</sup>	0.923 <sup>*</sup>
96	10.940± 0.058	y=2.8073x-2.0822	0.985 <sup>#</sup>	0.988 <sup>*</sup>

<sup>#</sup> indicates no significant difference between observed and expected values (P > 0.05)

<sup>\*</sup> indicate statistical significant values at 0.01 levels (P < 0.05)



**Figure 2:** Regression plot between Log concentration and probit Mortality at different exposure periods (P < 0.05).

**Table 4:** Effect of SDS on behavioral responses of *Tubifex tubifex* (HM: Hyperactive movement; CT: clumping tendency; MS: mucus secretion; WE: wrinkling effect; -: none; +: mild; ++: moderate; +++: strong) exposed to different concentration in different exposure period.

Behavioral Responses of <i>Tubifex tubifex</i>											
Time (h)	Behavioral Response	Dose (mg/l)									
		0 (Control)	5	10	15	20	25	30	35	40	45
24	HM	-	-	+	+	++	++	+++	+++	+++	+++
	CT	+++	+++	+++	++	++	+	+	-	-	-
	MS	-	-	-	-	+	+	++	+++	+++	+++
	WE	-	-	-	-	+	++	++	+++	+++	+++
48	HM	-	-	+	+	++	++	+++	+++	+++	+++
	CT	+++	+++	++	++	++	++	+	-	-	-
	MS	-	-	-	-	+	+	+	++	+++	+++
	WE	-	-	-	-	+	+	++	++	+++	+++
72	HM	-	-	+	+	+	+	++	++	++	+++
	CT	++	++	++	++	+	+	+	-	-	-
	MS	-	-	-	-	+	+	+	++	++	++
	WE	-	-	-	-	+	+	+	++	++	++
96	HM	-	-	-	+	+	+	+	++	++	+++
	CT	++	++	++	++	+	+	+	-	-	-
	MS	-	-	-	-	+	+	+	+	++	++
	WE	-	-	-	-	+	+	++	++	++	++

**Table 5:** Safe permissible limit of SDS to oligochaete worm *Tubifex tubifex*

Surfactant	96 h LC <sub>50</sub> (mg/l)	Method	AF	Safe Level(mg/l)
Sodium Dodecyl Sulphate	10.940	Edwards and Brown (1966)	0.4	4.376
		Burdick (1967)	0.1	1.094
		Sprague(1971)	0.1	1.094
		CWQC (1972)	0.01	0.1094
		IJC (1977)	5% of 96h LC <sub>50</sub>	0.01065
		CCREM (1991)	0.05	0.01065

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