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Studies on the toxicity of Alkylbenzene sulphonate to Zebra fish, *Danio rerio* Hamilton

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Abstract

Detergents are common household products used for cleaning of domestic materials. The 'after wash' of the detergents are either drained in to the aquatic environment such as ponds, lakes, rivers and streams or they find their way in to the aquatic environment by natural sewage. In the present study, the acute toxic effect of a detergent "Alkylbenzene sulphonate" (ABS) on *Danio rerio* was evaluated. For acute toxicity study, the fishes were exposed to different ABS concentrations (22, 24, 26, 28, 30, 32, 34, 36, 38 mg/l) for 12, 24, 48, 60, 72, 84 and 96hrs duration. The LC₅₀ values of the prepared concentration for 12, 24, 36, 48, 60, 72, 84 and 96 hrs were 36.427, 32.506, 32.681, 31.717, 29.675, 29.506, 28.225 and 27.310 respectively.

Keywords: *Danio rerio*, Detergent, domestic wastes, mortality, and toxicant.

1. Introduction

Water is the basis of life on the earth and a vital element for survival of human beings. Among the planet's renewable resources water has unique place, and is essential for sustaining all forms of life, food production, and economic development and for general Well-being. There is no substitute for water; a unique gift to mankind from nature [1]. The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades [2, 3]. Toxic substances can sometimes be lethal or semi-lethal [4]. Sublethal concentrations of toxicant may affect the behaviour of organisms and fitness of a natural population [5].

Water is polluted chiefly from two sources, human sewage waste and industrial waste [6]. One of the most common domestic wastes that commonly enter the aquatic ecosystem is detergent, which is a non-biodegradable chemical substance [7]. Linear Alkyl benzene Sulphonate (LAS) is the most widely used anionic surfactant in household and cleaning products that lowers the surface tension of water, enabling soils and stains to loosen and release from fabrics and surfaces. These anionic surfactants are reported to be acutely toxic to aquatic organisms [8, 9]. Contamination of natural water by detergents has become a matter of concern in recent years because of their large scale use in home and industrial applications, such as, washing powders, dye fasteners, formulation of shampoos, industrial and household cleansing agents, toothpaste, tooth powder and dispersing oil spills etc. [10, 11].

LAS is the primary cleaning agent used in many liquid and powder laundry detergents and speciality household cleaners at concentrations up to 25 percent of the total formulation. Surfactants are often referred to as the "engine" of the detergent system. During wastewater treatment, up to 99% of LAS are removed [12].

The detergents and surfactants are non-degradable pollutants accumulated in water bodies. These detergents enter the food web through uptake by vegetation, planktons, fishes and zooplanktons. Entry of detergents into aquatic system build up in the food-chain and are responsible for many hazardous effects and even death of the aquatic organisms, including fishes [13]. Fish is one of the most important non-target aquatic organisms affected by detergent pollution [14]. Fishes are very good biosensors of aquatic contaminants and bio-indicator species respond with great sensitivity to changes in the aquatic environment. Literature revealed that detergent related works on fish are still very meagre and limited to acute toxicity determination [15, 16, 17]. Saxena [18] reported the toxic effect of four commercial detergents (Two washing powders and two cakes) on behaviour, mortality and RBC counts of a freshwater fish *Gambusia affinis* and Guppy fishes *Poecilia reticulata* (Peters). The zebra fish *Danio rerio* belongs to the family Cyprinidae, the most species rich vertebrate family (Nelson,

1994). The name *Danio* derives from the Bengali name “dhani” meaning “of the rice field” (Talwar and Jhingran, 1991). *D. rerio* was first described by Francis Hamilton, a surgeon with the British East India Company, stationed principally in West Bengal at the beginning of the 19th century. The aim of this work was to study the mortality rate of *D. rerio* by exposing them to different concentrations of Alkyl benzene sulphonate, a common surfactant used in all the detergents.

2. Materials and Methods

The present study was carried out during the study period of 2012-2013 in the laboratory, Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil, Tamil Nadu.

2.1. Collection and acclimatization of the test fish

Healthy *D. rerio* fish were obtained from the fish farm at Azhagamandapam. They were acclimatized and maintained under laboratory condition (25-28 °C) for 15 days, fed with commercial fish-food prepared from “Dried Spirulina, Daphnia and Mysis”. The fish was acclimatized in large FRP tanks containing tap water. Care was taken to avoid any sudden changes in temperature, salinity and pH. The fish was acclimatized for about 15 days before the commencement of the experiments.

2.2. Toxicity bioassay

The toxicity bioassay studies were carried out for Alkyl benzene sulphonate. About 300 mg of the ABS powder was weighed and thoroughly mixed with tap water and made into 150 ml as a stock solution. Different concentration of stock solutions with a concentration of 22, 24, 26, 28, 30, 32, 34, 36,

38 mg/l were prepared by mixing with ordinary aerated tap water and made in to 1 litre in which 10 numbers of same size *D. rerio* fish were introduced, and for each set two replicates were maintained. The mortality of the fish was observed after 12, 24, 36, 48, 60, 72, 84 and 96 hours of exposure. The data was presented in the form of mortality table ^[19].

2.3. Statistical Analysis

The mortality response of the fish was further analysed using probit analysis ^[20]. Log dose and probability of mortality values were calculated. LC₅₀ values were derived through probit analysis and the upper and lower confidence intervals were calculated.

3. Result

After 12h exposure to Alkylbenzene sulphonate 20 percent mortality of *D. rerio* was recorded at a toxicant concentration of 32 mg/l. At a concentration of 34 mg/l the mortality was 30 percent. 50 percent and 60 percent mortalities were recorded at 36 mg/l and 38 mg/l respectively. Probit analysis of the toxicity response of *D. rerio* to Alkylbenzene sulphonate (Table 1) were used to find out nh LC₅₀ values and their upper and lower confidence intervals (Table 2). The LC₅₀ value for 12 h exposure was 36.427, the LCL 33.92 and UCL 39.08. X and Y values were 1.546 and 4.763 respectively.

After 84 h exposure to Alkylbenzene sulphonate 20 percent mortality of *D. rerio* was recorded at a toxicant concentration of 26 mg/l. At a concentration of 28 mg/l the mortality was 50 percent, 70 percent and 90 percent mortalities at 30 mg/l and 32 mg/l respectively. The LC₅₀ value for 84 h exposure was 28.225, the LCL 26.96 and UCL 29.51 and X and Y values were 1.458 and 5.164 respectively.

Table 1: Mortality response of *Danio rerio* to Alkylbenzene Sulphonate (ABS)

S. No	Concentration in mg/l	Mortality in hours							
		12h	24h	36h	48h	60h	72h	84h	96h
1	22								-
2	24								10
3	26						10	20	30
4	28				10	20	30	50	60
5	30		10	20	30	40	50	70	80
6	32	20	30	40	50	70	80	90	100
7	34	30	50	60	70	80	100		
8	36	50	80	90	100				
9	38	60	100	90	100				

In *D. rerio* exposed to Alkylbenzene Sulphonate at 24 mg/l concentration for 96 h, 10 percent mortality was recorded. 30 and 60 percent mortalities were occurred at the concentration of 26 mg/l and 28 mg/l. 80 and 100 percent mortalities were

recorded at the concentration of 30 mg/l and 32 mg/l respectively. The LC₅₀ value for 96 h exposure was 27.310, the LCL 26.18 and UCL 28.45 and X and Y values were 1.437 and 5.017 respectively.

Table 2: LC₅₀ values and their fiducially limits of Alkylbenzene Sulphonate (ABS)

S. No	Hours	LCL	LC ₅₀	UCL
1	12	33.92	36.427	39.08
2	24	32.35	32.506	33.49
3	36	31.40	32.681	33.98
4	48	30.46	31.717	32.98
5	60	29.28	29.675	30.66
6	72	28.35	29.506	30.68
7	84	26.96	28.225	29.51
8	96	26.18	27.310	28.45

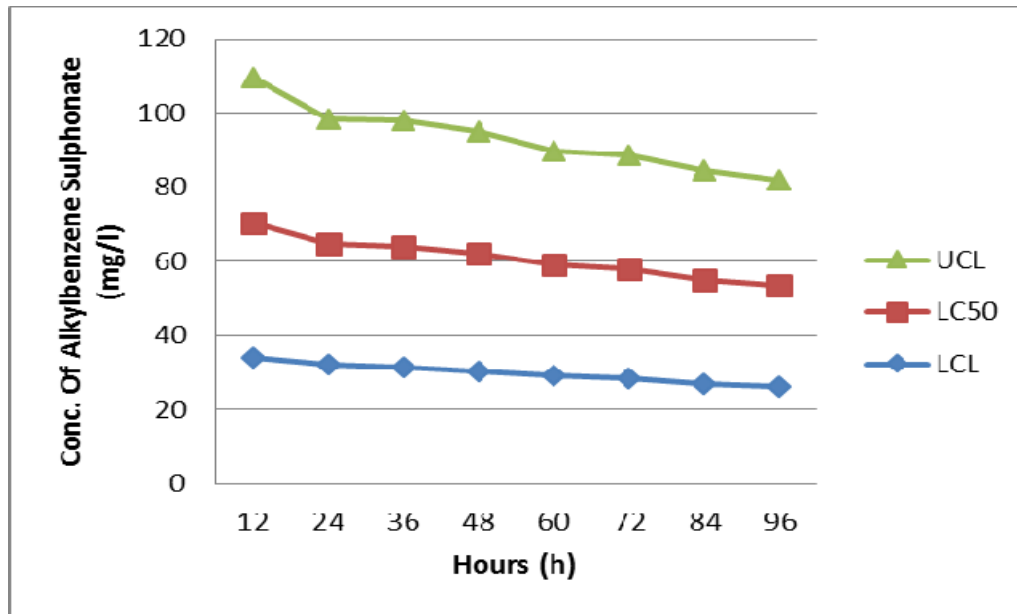


Fig 1: LC₅₀ values and their fiducially limits of Alkylbenzene Sulphonate (ABS)

4. Discussion

This study provided several important contributions to the field of environmental toxicity. Acute toxicity test was conducted to determine LC₅₀ values of Alkylbenzene sulphonate to the adult Zebra fish, *D. rerio*. Acute lethal toxicity test and the determination of LC₅₀ value is the most reliable test for assessing the potential adverse effect on aquatic life [21]. Further it is an important test to determine the concentration of a toxicant which may be allowed in receiving waters without adverse effects on living resources [22]. The response of *D. rerio* to different concentrations of alkylbenzene sulphonate was also studied and the results revealed that the sublethal, lethal concentration recorded for the alkylbenzene sulphonate is 22 and 38 mg/l respectively. The small changes observed in the fish in lower lethal and sublethal concentrations of the detergent effluent (0.01mg/l, 0.02 mg/l & 0.03 mg/l and 0.002 mg/l, 0.003 mg/l and 0.004 mg/l respectively) may be due to the avoidance behaviour of the test organisms to the detergent effluent, this conformed with the submission of Donaldson and Dye [23]. Who are of the opinion that, fish exposed to low concentration of toxicant do not reach the stage of exhaustion, rather they quickly become adapted to the stressor.

The stressful and erratic behaviour of the fish in this investigation gives a signal to respiratory impairment, and this may be due to the effect of the detergent effluent on the gills, and this finding is in agreement with the opinion of earlier reports of Adewoye *et al.*, Ayoola and Ogundiran *et al.* [24, 25, 17]. According to Kulakkattolickal and Kramer [26] at increased lethal and sublethal concentrations (0.04 mg/l & 0.05 mg/l and 0.005 mg/l and 0.006 mg/l respectively), the behavioural responses of the test organisms greatly increased and later the organisms become inactive and this is a normal situation in acute and sub-acute toxicity test, and the same finding was reported in the present study also. Warren and Ayoola [27, 25] reported that indiscriminate deposition of effluents into an aquatic systems might decrease the dissolved oxygen concentration leading to asphyxiation and may ultimately result into organ architectural degradation such as liver dysfunction.

In the present study LC₅₀ value of Alkylbenzene sulphonate to the *D. rerio* was found to be 36.43, 33.51, 32.68, 31.72, 30.68, 29.51, 28.23 and 27.31 mg/l at 12, 24, 36, 48, 60, 72, 84 and

96 hrs respectively (Table 2). The data indicated that a decrease in LC₅₀ concentration is associated with increase in duration of exposure. The value of 27.310 as the 96 hours LC₅₀ recorded in this investigation makes the toxicant highly lethal for fish and this value is within the range of 0.4 to 40.00 mg/l reported by Abel [28] on synthetic detergents to be acutely toxic to fish. Maruthanayagam [29] reported 24 hrs LC₅₀ value of detergent to *Macrobrachium lamarrei* was 0.5%. Shingadia and Sakthivel [30] reported 96 hours LC₅₀ value of wheel detergent for *Lamellidens marginalis* (Lamarck) was 400ppm whereas studies showed that the gill damage is the most obvious acute toxic effect; the immediate cause of death may be due to asphyxiation, but detergents may also be toxic internally. Sublethal effects include retardation of growth, alteration of feeding behaviour and inhibition of chemoreceptor organs [14]. It was also observed that the mortality rate of the fish increased with the increased time of exposure to Alkylbenzene sulphonate. The lethal concentration (LC₅₀) was inversely proportional to duration of exposure [31]. The mortality rate of *D. rerio* remained directly proportional to duration of exposure and concentration of the Alkylbenzene sulphonate. Similar observations were made by Gabriel and Okey [32] in catfish hybrid exposed to textile industrial effluent.

5. Conclusion

Conclusively, this study has been able to establish the fact that, exposure of *D. rerio* to Alkylbenzene sulphonate can induce various toxicological effects, which dependent on the period of exposure and concentration of toxicant.

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