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Acute Toxicity Assessment of Dyeing Industry Effluent in Fish: Investigating Time-Dependent Lethal Concentrations

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ABSTRACT

This article highlights the importance of toxicity studies in assessing the adverse effects of agents on living organisms, focusing on product safety and environmental protection. It discusses the Indian regulatory landscape, including the Central Drugs Standard Control Organization (CDSCO) and Schedule Y of the Drugs and Cosmetics Rules, as well as ethical oversight by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). It also presents a materials and methods section for an acute toxicity study involving fish exposed to raw dyeing industry effluent, focusing on determining the 96-hour LC50 using Probit analysis. The results show that even initially "safe" concentrations can become lethal over prolonged periods, emphasizing the relevance of acute toxicity data for regulatory bodies and risk assessment.

Keywords: *Acute toxicity, Environmental toxicology, Aquatic pollution, Sublethal effects, Effluent impact*

1. INTRODUCTION

Toxicity studies, also known as toxicology testing or safety assessment, are scientific investigations designed to determine the adverse effects of chemical, biological, or physical agents on living organisms. The goal is to understand the step to which a substance can cause harm, given a certain exposure duration, route of exposure, and concentration. These studies are crucial for ensuring the safety of new drugs, chemicals, pesticides, food additives, cosmetics, and environmental pollutants [1]. The fundamental purposes of toxicity studies are to: Determine the safety profile of a substance before it is introduced to humans or released into the environment. Identify the specific adverse effects a substance can cause (e.g., organ damage, carcinogenicity, reproductive issues). Establish the relationship between the dose of a substance and the severity of its effects. This helps determine safe exposure levels and the maximum tolerated dose (MTD). Identify which organs or systems are most susceptible to the toxic effects of a substance. Understand how a substance causes its toxic effects at a molecular or cellular level. Provide essential data required by regulatory bodies (e.g., FDA, EMA, CDSCO in India) for the approval and registration of new products. Generate data that allows for the assessment of potential risks to human health and the environment, leading to the development of regulations and safety guidelines [2,3].

Toxicity studies are typically classified based on the duration of exposure and the type of effect being investigated. They are performed using various methods, including *in vivo* (using whole animals), *in vitro* (using isolated cells or tissues), and *in silico* (computer simulations). In India, toxicity studies, particularly for pharmaceuticals, are primarily governed by the Central Drugs Standard Control Organization (CDSCO), which operates under the Ministry of Health and Family Welfare. The key guiding document is Schedule Y of the Drugs and Cosmetics Rules, 1945 (as amended by the New Drugs and Clinical Trials Rules, 2019) [4].

These papers lay down the detailed requirements for preclinical (animal) toxicity studies that must be conducted before a new drug can enter human clinical trials. They specify:Acute, repeated-dose (e.g., 14-day, 28-day, 90-day, chronic), reproductive, developmental, genotoxicity, carcinogenicity, local toxicity, allergenicity/hypersensitivity, and safety pharmacology studies. Typically at least two mammalian species (one rodent, one non-rodent). Usually three dose levels (high, mid, low) plus a control group. The high dose should elicit some toxicity, while the low dose should ideally be a NOAEL. Should be the same as the intended clinical route. Comprehensive clinical observations, body weight, food/water consumption, clinical pathology (hematology, clinical chemistry, urinalysis), gross pathology, organ weights, and

histopathology. Specified based on the proposed duration of human clinical trials and eventual marketing. All non-clinical safety studies (toxicity studies) must be conducted in GLP-compliant laboratories. GLP ensures the quality, integrity, and reliability of the data. India has a National GLP Compliance Monitoring Authority (NGCMA) under the Department of Science & Technology [5].

The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the Ministry of Fisheries, Animal Husbandry and Dairying, governs the ethical conduct of animal experiments. All toxicity studies involving animals require prior approval from the Institutional Animal Ethics Committee (IAEC) which is registered with CPCSEA. The "3Rs" principle (Replacement, Reduction, Refinement) is strongly encouraged. Besides Schedule Y, other specific guidelines may apply depending on the product type (e.g., for biosimilars, vaccines, medical devices, or Ayurvedic/traditional medicines, for which the Ministry of AYUSH might have specific guidelines). For industrial chemicals and pesticides, other regulatory bodies like the Central Insecticides Board & Registration Committee (CIB&RC) and the Ministry of Environment, Forest and Climate Change (MoEFCC) oversee toxicity testing requirements, often referencing OECD guidelines.

2. MATERIALS AND METHOD

Upon arrival at the laboratory, fish will undergo a minimum 7-day acclimation period in aerated glass holding tanks containing dechlorinated municipal tap water, maintained at consistent conditions (temperature: 25±2∘C; pH: 7.5±0.5; 12h light:12h dark photoperiod). During this period, fish will be fed daily, with feeding discontinued 24 hours prior to the start of the experiment. Only fish exhibiting uniform age, length (± 10% of mean), and weight (± 10% of mean), and showing no signs of stress or disease, will be selected for the study. Raw dyeing industry effluent will be collected from a designated source, stored refrigerated at 4∘C, and allowed to reach room temperature before use. A series of at least five concentrations of the effluent will be prepared using the dechlorinated tap water as dilution water, along with a control group (0% effluent), in appropriately sized glass test chambers, ensuring adequate loading density.

For each concentration and the control, 10 selected fish will be gently introduced into the respective chambers. Key physico-chemical parameters of the test solutions, including temperature, pH, dissolved oxygen (maintained above 5 mg/L with gentle aeration), and conductivity, will be measured at 0 hours and at 12-hourly intervals throughout the 120-hour exposure period. Mortality will be assessed and recorded at 12-hourly intervals, with dead fish promptly removed; any observable sublethal effects will also be noted. The collected mortality data will then be subjected to statistical analysis, primarily Probit analysis, to determine the 96-hour LC50 (Lethal Concentration 50%) value and its 95% confidence intervals, which will serve as the principal index of the effluent's acute toxicity. Furthermore, to investigate sublethal impacts, additional experiments will be performed using concentrations equivalent to one-fourth (1/4th) and one-tenth (1/10th) of the determined 96-hour LC50, with observations focusing on behavioral and physiological changes. Finally, appropriate toxicity curves, including concentration-mortality and time-mortality graphs, will be constructed to graphically represent the toxic effects of the effluents. All experimental procedures will adhere to GLP principles and ethical guidelines for animal care, ensuring data integrity and humane treatment of test organisms.

3. RESULT AND DISCUSSION:

Table 1. Effiluent analysis of Dyeing Industry

Hours of observation	LCL	LC_{50}	UCL
12	39.71	44.8	50.56
24	34.249	37.402	40.754
36	31.22	34.355	37.392
48	27.699	30.414	33.36
60	24.378	27.283	30.425
72	21.249	23.726	26.90
84	18.923	21.433	24.245
96	16.300	18.648	21.63
108	14.684	16.730	19.040
120	11.96	14.07	16.59

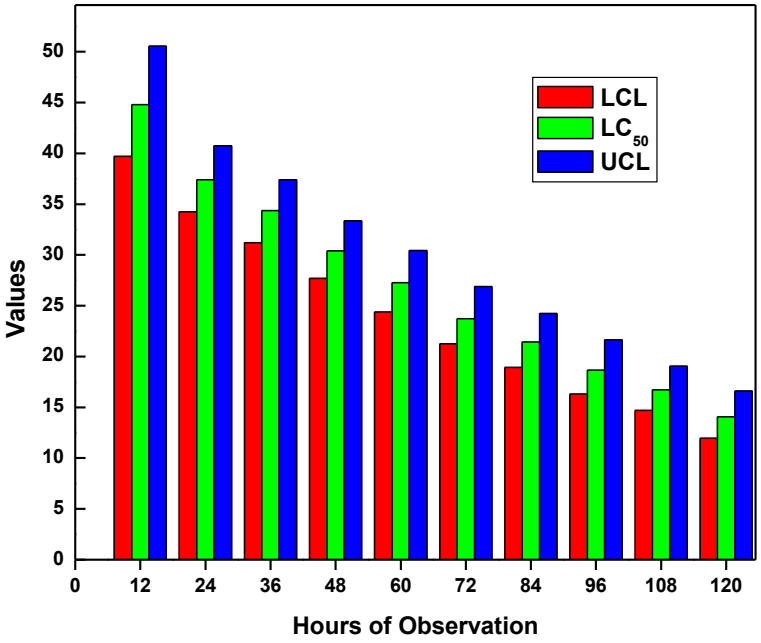

Fig 1. Shows the Hours of Observation Values.

The provided data clearly illustrates a fundamental principle of toxicology: time-dependent toxicity. This concept posits that the effect of a toxic substance is not solely determined by its concentration, but also significantly by the duration of exposure, a cornerstone of dose-response relationships in toxicology [6]. The Lethal Concentration 50 (LC50), representing the concentration of a substance estimated to be fatal to 50% of a test population under specific experimental conditions, is observed to consistently decrease as the duration of observation increases [7]. This underscores a critical aspect of toxicological assessment, where the threshold for a lethal effect diminishes with prolonged contact [8]. Specifically, the LC50 value for the substance under study demonstrated a marked drop from 44.8 at 12 hours of observation to a significantly lower 14.07 after 120 hours [9]. The values are shown in table.1 and the comparison bar is illustrated in **Figure.1**. This clear trend, where a lower concentration becomes lethal over a longer exposure period, is a typical and expected finding in acute toxicity assessments. It critically underscores that even concentrations initially considered "safe" or non-lethal for shorter durations can indeed become lethal if exposure is prolonged. Furthermore, the accompanying Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL) also consistently narrow and shift downwards with increased observation time. This refinement in the confidence intervals indicates a more precise and accurate statistical estimation of the LC_{50} as the toxic effect fully manifests and the biological system has more time to respond to the stressor [10].

This phenomenon of time-dependent toxicity is crucial for understanding and predicting the potential hazards of chemicals in various contexts. In environmental settings, for instance, aquatic organisms continuously exposed to pollutants may succumb to concentrations far lower than those found to be acutely lethal in short-term laboratory tests, highlighting the long-term ecological risks posed by persistent [11]. Similarly, in occupational health, even exposures at seemingly low levels can become dangerous with chronic or prolonged contact, emphasizing the need for stringent long-term exposure limits and comprehensive risk assessment beyond acute effects. Therefore, acute toxicity studies, like the one presented, provide invaluable baseline data for regulatory bodies and risk assessors. This data is essential to establish safe exposure limits and to accurately classify the hazard potential of substances, always considering the dynamic interplay between the concentration of a substance and the duration of exposure. While LC_{50} is undeniably a key metric for understanding acute toxicity, it is essential to remember that it is primarily a statistical estimate of lethality [12]. It does not fully capture the complex, non-lethal effects, sub-lethal impacts, or chronic health repercussions of a substance, which often necessitate further, more comprehensive, and longer-term toxicological investigations to fully characterize the risk profile.

4. CONCLUSION

The study shows that dyeing industry effluent on fish decreases with increasing exposure duration, highlighting the time-dependent nature of toxicity. This highlights the dynamic relationship between substance concentration and exposure time, highlighting that even low concentrations can become lethal over prolonged periods [13]. The methodology, adhering to

GLP principles and ethical animal care guidelines, provides reliable baseline data for acute toxicity assessment. The findings are crucial for regulatory bodies in India to formulate more robust environmental protection strategies and industrial discharge norms [14].

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