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# Acute Toxicity and Genotoxicity of Sublethal Concentrations of Sodium Lauryl Sulfate and Sodium Tripolyphosphate in *Clarias gariepinus* (The African Sharptooth Catfish)

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**Abstract**
Personal care products (PCPs) are emerging pollutants which have been detected in aquatic environments and are potentially toxic to aquatic organisms. The acute toxicity of two (2) PCPs - sodium lauryl sulfate (SLS) and sodium tripolyphosphate (STPP) to fingerlings of *Clarias gariepinus* (the African Sharptooth Catfish) was evaluated over a period of 96 h. Furthermore, genotoxic (erythrocytic micronuclei frequencies) evaluations were conducted in juveniles of *C. gariepinus* exposed to sublethal concentrations of the test chemicals over a period of 28 days. The median lethal concentration of SLS and STPP to *C. gariepinus* was 14.75 mg/L and 861 mg/L respectively with SLS being x59 more toxic than STPP to *C. gariepinus*. Micronuclei frequencies in SLS-exposed fishes were significantly higher ($p<0.05$) at day 14 (3.44±1.08 ‰) and day 28 (5.78±1.18 ‰) compared to the control (day 14- 0.78±0.22 ‰; day 28- 1.56±0.29 ‰). However, in STPP-exposed fishes, micronuclei frequencies were significantly higher ($p<0.05$) at day 28 (4.11±0.89 ‰) only compared to the control. The study showed that non-target aquatic animals such as *C. gariepinus* might be at risk from exposure to these chemicals in surface waters from diffuse sources with SLS posing a higher risk than STPP. Targeted environmental management and advocacies are recommended to promote sustainability of life below water (United Nations Sustainable Development Goal 14).

**Keywords:** Catfish, DNA damage, Sodium Dodecyl Sulphate, Sustainable Development, Environmental pollutants

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## INTRODUCTION

Personal care products (PCPs) are classified as emerging pollutants due to their ubiquity in the environment and toxic effects at low concentrations (Homem *et al.*, 2014; Sogbanmu *et al.*, 2019). PCPs are products that are used to beautify or improve the quality of daily life such as moisturizers, toothpastes, detergents, deodorants and shampoos. Being used by humans, they are often discharged into sewage systems following use (Boxall *et al.*, 2012). They are discharged into surface waters through point sources such as sewage systems or through non-point (diffuse) sources (runoff) (Ying, 2006). Surfactants such as

sodium lauryl sulfate (SLS) and sodium tripolyphosphate (STPP) are anionic compounds that are often used as emulsifying agents in cleaning products such as laundry detergents, spray cleaners, dishwasher detergents, among others (Cserhati *et al*., 2002; Bondi *et al*., 2015). SLS has rapid biodegradation rates under aerobic and anaerobic conditions (Singer and Tjeerdema, 1993).

The effects and risk of PCPs in the environment have been elucidated in the last two decades (Boxall *et al*., 2012; Yao *et al*., 2018; Sogbanmu *et al*., 2019; Nagar *et al*., 2020). Several chemicals used in the production of PCPs are biologically active compounds that are designed to interact with specific pathways and processes in target humans and animals (Boxall *et al*., 2012). The acute toxicity of PCPs such as triclosan, triclocarban and effluents from PCPs manufacturing companies have been observed in the African catfish (*Clarias gariepinus*) at low concentrations (Jimoh, 2018; Sogbanmu *et al*., 2019). Median lethal values of 15.1 mg/L of SLS have been recorded for the Eastern mosquito fish (*Gambusia holbrooki*) (Nunes *et al.,* 2005) while the 96 h $LC_{50}$ value of SLS against *Oncorhynchus mykiss* (Rainbow Trout) has been reported to be 24.9 mg/L (HSDB, 2015). Biomarkers at nuclear and cellular levels of organisation in fish species have been shown to provide an indication of sublethal risk of exposure to or effects of toxicants (Mumuni and Sogbanmu, 2018). Micronuclei frequencies in exposed organisms serve as biomarkers of genotoxic effects which if not abated could lead to more deleterious or lethal effects at the organismal and higher levels of biological organisation (Sogbanmu, 2015).

The African catfish, *Clarias gariepinus* is a species of air-breathing catfish that is omnivorous (Skelton, 1993). It is an ecologically and commercially important freshwater fish that is found in the wild and one of the most cultured fish species in several African countries especially Nigeria (Esenowo and Ugwumba, 2010). Though, SLS and STPP have been reported to be biodegradable (Singer and Tjeerdema, 1993; Bondi *et al*., 2015), their potential acute and chronic toxicities to indigenous fish species like *C. gariepinus* are little known. Consequently, the aim of this study was to evaluate the acute toxicity and genotoxic effects of sublethal concentrations of sodium lauryl sulfate (SLS) and sodium tripolyphosphate (STPP) to fingerlings and juveniles of *C. gariepinus*. The findings will contribute to toxicity data on SLS and STPP to different fish species and ecological contexts. Furthermore, the results will provide ecological risk information for environmental management in order to sustain life in water (United Nations Sustainable Development Goal 14).

## MATERIALS AND METHODS

### Test chemicals

The test chemicals were personal care products namely sodium lauryl sulfate (SLS) (also known as sodium lauril sulfate or sodium dodecyl sulfate (SDS)) and sodium tripolyphosphate (STPP). They were obtained from the Bariga market, Lagos, Nigeria. SLS has a grain-like appearance and is white in colour. STPP is a sodium triphosphoric acid. It is a crystal powder and is white in colour. The test chemicals were used singly and in a non-additive manner.

### Test animal collection and acclimatization

Fingerlings (weight range: 7-11 g; length range: 5.0-6.5 cm) and juveniles (weight range: 16.3-30 g; length range: 14.5-18.3 cm) of *Clarias gariepinus* (Chordata, Osteichthyes, Siluriformes, Clariidae) were procured from the Department of Marine Sciences, Faculty of Science, University of Lagos, Nigeria. The fish were transported in an aerated plastic to the Department of Zoology laboratory where they were transferred to a transparent rectangular 60 L plastic tank. The fish were acclimatized to laboratory conditions for five (5) days. During this period, the fingerlings and juveniles were fed once daily with a commercial fish feed (Skretting), which typically contains a mix of marine (fishmeal and fish oil) and vegetable (wheat, soy, rapeseed, sunflower and faba) pellets of 1.5 mm and 3 mm respectively. The water

(dechlorinated) in the holding tanks was changed daily to dispose of uneaten food and faeces. The fish were starved for 24 h prior to the bioassays (Sogbanmu and Otitoloju, 2014).

**Preparation of test media**

The test chemicals used were in solid form and were both soluble in water. For SLS, a working stock solution was made by dissolving 1 g of the chemical in 1 L of dechlorinated water to make 1 g/L. A working solution of 10 g/L of the chemical was made for STPP.

**Acute toxicity studies with fingerlings of *Clarias gariepinus* exposed to sodium lauryl sulfate (SLS) and sodium tripolyphosphate (STPP)**

Range finding tests were conducted before the commencement of the definitive tests in order to determine the concentration range of the toxicants against *C. gariepinus* fingerlings. Following the range finding tests, four (4) fingerlings of similar sizes were randomly selected from the stock tank into the test media containing varying concentrations of SLS (3, 6, 12, 14 and 24 mg/L), STPP (300, 450, 600, 750 and 900 mg/L) and untreated control in triplicates. The bioassays tanks were 3L plastic tanks. A static non-renewable bioassay protocol was employed in which test media was not renewed throughout the duration of the experiment (OECD, 1992). Mortality was assessed every 24 h over a period of 96 h.

**Genotoxicity studies (micronucleus assay) with juveniles of *Clarias gariepinus* exposed to sublethal concentrations of sodium lauryl sulfate (SLS) and sodium tripolyphosphate (STPP)**

Four (4) *C. gariepinus* juveniles of similar sizes in triplicates were exposed to a sublethal concentration (10% 96 h $LC_{50}$ values) of the test compounds (SLS- 1.47 mg/L; STPP- 86 mg/L) and an untreated control (dechlorinated water alone) in 5 L plastic tanks containing 3 L of dechlorinated water for 28 days. A non-static renewable bioassay protocol was employed in which the test media was renewed every 48 h throughout the duration of the experiment. At post-exposure periods of 14 and 28 days, juveniles were randomly selected from the test media including control and blood samples were drawn for micronucleus assay (Mumuni and Sogbanmu, 2018). Briefly, the peripheral blood was drawn from the caudal vein of the fishes with a 2 mL syringe. A smear was made on a clean glass slide and fixed with 100 % ethanol for 5 min, air dried, stained with May-Grunwald and then Giemsa. It was then washed with distilled water and allowed to dry. The slides were staged and analysed for 1000 cells/individual micronuclei. Nuclear abnormalities like micronuclei in the blood cells were observed and recorded. The criteria for identifying micronuclei in the erythrocytes were the following features: similar intensity of stained micronucleus to main nucleus, smaller diameter to the main nucleus, having round nuclear membrane, no connection to the main nucleus, no overlap with the main nucleus and it is found within the cytoplasm (Mumuni and Sogbanmu, 2018; ). This study followed the principles in AVMA guidelines for the care and use of animals in research (AVMA, 2013).

**Statistical analysis**

Probit analysis was conducted using SPSS version 20.0 on the acute toxicity data to derive the median lethal concentration (96 h $LC_{50}$). Micronuclei results are presented as mean±standard error. One Way Analysis of Variance (ANOVA) with Dunnett Post-Hoc test was used to test for significant difference between treatment means at days 14 and 28 using SPSS version 20. Significant difference between treatments were set at $p<0.05$.

## RESULTS

**Relative acute toxicity of sodium lauryl sulfate (SLS) and sodium tripolyphosphate (STPP) against fingerlings of *Clarias gariepinus***

The median lethal concentration (96 h $LC_{50}$) of SLS and STPP when tested on *Clarias gariepinus* fingerlings were 14.75 mg/L and 861 mg/L

respectively (Table 1). On the basis of toxicity factor, SLS was observed to be approximately x59 more toxic than STPP on exposure to *C. gariepinus* fingerlings (Table 1).

**Table 1:** Relative acute toxicity of sodium lauryl sulfate (SLS) and sodium tripolyphosphate (STPP) against *Clarias gariepinus* fingerlings

| Treatment | 96 h $LC_{50}$ (mg/L) | Slope ± S.E. | Probit Line Equation | D.F. | T.F. |
|---|---|---|---|---|---|
| SLS | 14.75 | 2 ± 0.463 | Y = -2.3+2x | 3 | 58.9 |
| STPP | 861 | 6 ± 1.486 | Y = -16.4+6x | 3 | 1 |

SE = Standard error; 96 h $LC_{50}$ = median lethal concentration at 96 h; DF = Degrees of freedom

Toxicity Factor (TF) = $\frac{\text{96 h}LC_{50}\text{ of least toxic compound}}{\text{96 h}LC_{50}\text{ of most toxic compound}}$

**Genotoxicity (micronucleus assay) in *Clarias gariepinus* juveniles exposed to sublethal concentrations of sodium lauryl sulfate (SLS) and sodium tripolyphosphate (STPP)**

In *C. gariepinus* juveniles exposed to sublethal concentrations of SLS, there was a significant increase ($p<0.05$) in erythrocytic micronuclei at day 14 (3.44±1.08 ‰) and day 28 (5.78±1.18 ‰) compared to the control (day 14 - 0.78±0.22 ‰; day 28 - 1.56±0.29 ‰) (Table 2). Micronuclei in STPP-exposed fishes were significantly higher ($p<0.05$) at day 28 (4.11±0.89 ‰) only compared to the control (Table 2). Micronuclei in SLS-exposed fishes were generally higher than those in STPP-exposed fishes at days 14 and 28 being significantly higher at day 14 only (Table 2).

**Table 2:** Genotoxic test (Micronucleus assay) results for *Clarias gariepinus* juveniles at days 14 and 28 exposed to sublethal concentrations of sodium lauryl sulfate (SLS) and sodium tripolyphosphate (STPP)

| Treatment (mg/L) | Average MN±SE (‰) Day 14 | Average MN±SE (‰) Day 28 |
|---|---|---|
| Control | 0.78±0.22 | 1.56±0.29 |
| SLS (10% of 96 $hLC_{50}$) - 1.5 | 3.44±1.08* | 5.78±1.18* |
| STPP (10% of 96 $hLC_{50}$) - 86 | 1.22±0.52 | 4.11±0.89* |

N= 9000 cells; MN – Micronuclei; SE – Standard Error

**DISCUSSION**

Data from acute toxicity studies are of biological and ecological importance as they can be used to determine application factors for derivation of water quality guidelines (Otitoloju *et al.*, 2018).

The median lethal concentration of SLS observed in this study is similar to the findings of Nunes *et al.* (2005) for SLS against *G. holbrooki* (15.1 mg/L). However, it is almost twice as toxic to *O. mykiss* (96 h $LC_{50}$ – 24.9mg/L). Other studies have shown the acute toxicity of SLS after 96 h to range from 1 to 13.9 mg/L confirming its moderate toxicity to aquatic life (Abel, 1974; Lewis, 1991, 1992; Warne and Schifko, 1999; Zheng *et al.*, 2006). There is a paucity of data on the acute toxicity of STPP to fish species except for data on the 48 h $LC_{50}$ value of 1850 mg/L to zebrafish (Dion, 1985) and 24 h $LC_{50}$ of 500 mg/L to trout (Kastner *et al.*, 1983). Thus, our findings on the 96 $hLC_{50}$ value of 861 mg/L for STPP to *C. gariepinus* are probably the first report for this

fish species or other species in the last three (3) decades. The low toxicity (>100 mg/L) of STPP as observed in this study may be the reason for the paucity of toxicity studies for the surfactant.

Genetic damage in fish cells could play a major role in decreasing the fitness of fish populations with short- and long-term consequences on their survival (Mumuni and Sogbanmu, 2018). The induction of micronuclei in peripheral erythrocytes of *C. gariepinus* juveniles exposed to sublethal concentrations of SLS corresponds with its high acute toxicity to *C. gariepinus* fingerlings. This finding corroborates the observation that chronic toxicity of anioinic surfactants are evident at concentrations as low as 0.1 mg/L (Lewis, 1991). The observed significant and persistent increase in micronuclei induction from day 14 to 28 disagrees with the reported rapid biodegradation of the SLS under aerobic and anaerobic conditions (Singer and Tjeerdema, 1993; Bondi *et al*., 2015). Though, its biodegradability is reported to be subject to factor such as temperature, sunlight, pH, aeration and biological activity (Marchesi *et al.* 1991 and Rebello *et al.* 2014). Similarly, the differential toxicity of STPP compared to SLS as observed in the low and non-significant induction of micronuclei is related to its observed low toxicity to *C. gariepinus* fingerlings. However, the significant induction in micronuclei by day 28 suggests that long term exposure to near environmentally relevant concentrations of the compound might pose a risk to resident aquatic organisms. Thus, the postulation that surfactant concentration of 0.5 mg/L of natural water would be essentially nontoxic to fish (Abel, 1974; Bondi *et al*., 2015) might no longer hold true.

## CONCLUSION

This study has shown the genotoxic effects of long-term exposure sublethal concentrations of SLS and STPP on *C. gariepinus* which can serve as biomarkers of effects of these compounds. For the sustenance of life in water (United Nations Sustainable Development Goal 14), there is an urgent need to improve wastewater treatment, management measures and intervention to reduce runoff from diffuse sources. Further studies are recommended to elucidate other biomarkers of toxicity at various levels of biological organisation in different animal species for holistic risk evaluation. Environmental regulatory agencies are encouraged to conduct periodic monitoring of small to medium scale industries which discharge these surfactants indiscriminately into the environment. Environmental education and advocacy about effects on fisheries and consequently human health through fisheries consumption and groundwater contamination should be conducted for a better informed public.

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