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Comparative acute toxicity of local detergents (Omo and Ariel) on fingerlings of the *Clarias gariepinus*♀ x *Heterobranchus longifilis*♂ hybrid

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Abstract. The acute toxicity of local detergents - Omo detergent (Unilever Nigeria Plc.) and Ariel detergent (Procter & Gamble Nigeria Limited) - were compared using fingerlings of *Clarias gariepinus* ♀ *x Heterobranchus longifilis* ♂ hybrid (*Heteroclarias*), in a 96 hour bioassay. After a series of range finding tests, the fishes were exposed to concentrations of 0.00 ppm, 20 ppm, 30ppm, 40 ppm, 45 ppm and 50 ppm of each detergent for 96 hours. The Median Lethal Concentrations (LC_{50}) values for the detergents ranged between 33.03–35.19 ppm and 37.43–39.79 ppm for Omo and Ariel, respectively. Manifestation times decreased from 62–14 and 70–14 hours; overturning times decreased from 80–16 and 92–20 hours, while survival times decreased from 96–17 and 96–23 hours for Omo and Ariel, respectively, with increasing concentrations of the toxicants. Respiratory disturbances, loss of righting balance, lethargy and sudden fish death were observed in the exposed fishes. There was a strong concentration-mortality relationship for toxicants, yielding a strong positive correlation co-efficient, r^2, of 0.9925 and 0.9882, respectively for Omo and Ariel detergents. The t-test analysis showed significant difference ($p < 0.05$) in manifestation time of Omo and Ariel detergents at 50 ppm concentrations. But there were no significant differences ($p > 0.05$) recorded in other concentrations. There were no significant differences ($p > 0.05$) in overturning and survival times of Omo and Ariel detergents in all the concentrations. The present study shows that Omo detergent with a lesser mean LC_{50} value of 34.11 ± 1.08 could be more toxic than Ariel with a mean LC_{50} value of 36.66 ± 1.1. Although there was no statistically significant deference between their LC_{50s} ($p > 0.05$), it was concluded that effluents containing these detergents must not be discharged indiscriminately into water bodies in order to avoid harm to fish and other aquatic life.
Key Words: ecotoxicological studies, water pollution, surfactants, fish, LC_{50} survival time, manifestation time, overturning time.

Introduction. In recent times in Nigeria and other developing nations, pollution of water resources has become a serious problem. This has been largely due to the rapid increase in human population and the proliferation of industries which have resulted in the discharge of large amounts of effluents and wastes into aquatic environments where they degrade the normal flora and fauna of the ecosystem (Adewoye et al 2005).

Human and ecological disorder experienced in industrial settlements as a result of improper disposal of chemicals such as detergent effluents calls for careful surveillance on the state of the environment. Only few chemicals have been ecologically tested in Nigeria for safety in spite of their environmental and ecological impacts (Avoajah & Oti 1997).

Presently, the Federal Government of Nigeria is emphasizing the need for adequate environmental protection in all technological and socio-economic development projects by strictly asking industrial operators to sustainable manage the disposal of chemicals such as detergents in the natural environment (DPR 2002).

Detergents are cleaning products derived from synthetic organic chemicals. The cheapness of detergent production from petrochemical sources with its ability to foam when used in acid or hard water gives it an advantage over soaps (Okpokwasili & Nwabuzor 1988).

Common detergents used in Nigeria include Omo produced by Unilever Nigeria Plc, Ariel produced by Procter & Gamble Nigeria Limited, etc. and they are composed of

surface-active agents or surfactants, bleach, builders, foam stabilizers, perfumes, enzymes, optical brighteners, etc. of which the most active components are the surfactants (Avoajah & Oti 1997).

Fishes are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution (Kock et al 1996). The hybrid catfish, *Clarias gariepinus* ♀ x *Heterobranchus longifilis* ♂ (*Heteroclarias*) (Figure 1) is hardy and can tolerate both well-oxygenated as well as poorly oxygenated waters, hence suitable for use as a biological indicator in this ecotoxicological study.

This study was conducted to assess the potential toxic effects of detergents on fishes and other aquatic life, by determining the $LC_{50,}$ as well as the manifestation, overturning and survival times of the hybrid fingerlings when exposed to various concentrations of the detergents used.

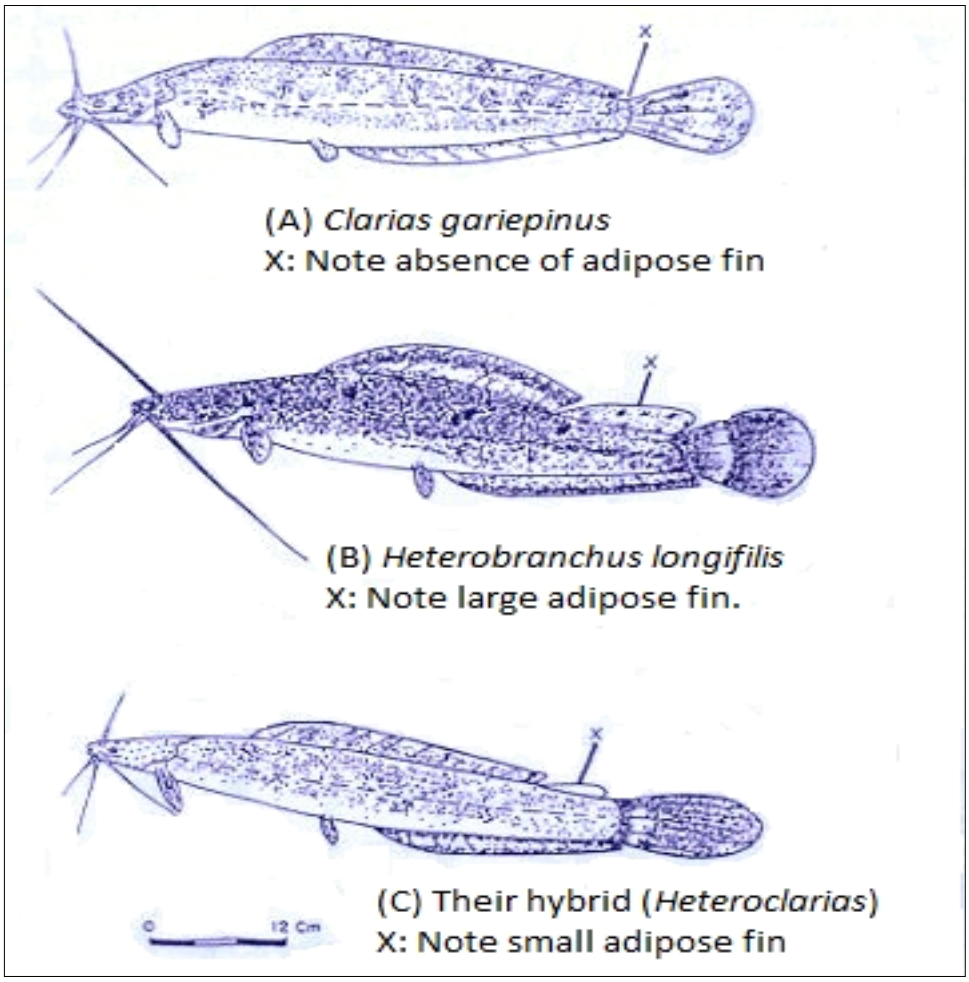

Figure 1. The adult fish of *Clarias gariepinus, Heterobranchus longifilis,* and their hybrid *Heteroclarias* (original drawing).

Material and Method. The study fish (Hybrid of *Clarias gariepinus* ♀ *x Heterobranchus longifilis*♂) fingerlings were obtained from the Premier Fish Farm in Nsit Ubiom Local Government Area of Akwa Ibom State Nigeria in January 2013, and transported to the laboratory in the Department of Zoology and Environmental Biology University of Calabar.

Two (2) locally made detergents Omo (manufactured by Unilever Nigeria Plc) and Ariel (manufactured by Procter & Gamble Nigeria Limited), were tested for their toxicities.

Ten (10) milligrams each of Omo and Ariel detergents were weighed using a sensitive weighing balance in the Chemical pathology Laboratory of the University of Calabar Teaching Hospital. Stock solutions of these test substances (detergents) were then prepared by dissolving each 10 mg in 1000 ml of clean and aerated water. Range

finding tests were conducted for Omo and Ariel detergents prior to the experiment. From the stock solutions of the toxicants, appropriate graded concentrations were made by serial dilutions. Five toxicant concentrations 20 ppm, 30 ppm, 40 ppm, 45 ppm, 50 ppm and a control were tested on the study fish. Test concentrations were spaced at logarithmic intervals to include one concentration showing 100% mortality, another with no mortality and other concentrations with partial mortalities. This allows for probit transformation of mortality data according to the method of Finney (1971). Tests were conducted in rectangular glass aquaria 30 cm x 60 cm x 30 cm cubic capacity, 3/4 filled with well aerated tap water. Ten (10) fishes were used for each test concentration of the toxicants with two replicates including the controls. Observations were made at intervals of 3 hours; dead fishes were removed and recorded.

Death was said to have occurred when a fingerling was motionless upon prodding with a glass rod. Time-effect relationships such as manifestation times (the time taken for the toxicant to show an effect on the fingerlings), overturning times (the time between the introduction of the toxicants and the loss of equilibrium of fingerlings) and survival times (the time between the introduction of the toxicant and the death of the fingerlings) were recorded accordingly.

The mortality data was subjected to probit transformation following the method of Finney (1971). The significance of the slope was tested by student's t-test.

Results

Manifestation time. Manifestation times for both Omo and Ariel detergents were observed to decrease with increase in concentrations of the toxicants. Fingerlings of the study fish exposed to 20, 30, 40, 45 and 50 ppm of Omo detergent showed mean manifestation times of 62, 28, 16, 14.5 and 14 hours, respectively, while those of Ariel detergent showed mean manifestation times of 70, 46, 22, 19 and 14 hours, respectively (Tables 1 and 2). Reduction in manifestation times was more gradual at higher concentrations than at lower concentrations in both toxicants. Compared to test individuals in the control tanks, where these test organisms were observed to be moving in groups, responses observed during manifestation times in toxicant treated individuals included hyperactivities, sudden bursts of movement, faster than normal breathing, rigorous movement from bottom to surface of tanks, etc.

Overturning time. Overturning times were observed to decrease with increase in concentrations of Omo and Ariel detergents. *Heteroclarias* exposed to 20, 30, 40, 45 and 50 ppm of Omo detergent showed mean overturning times of 89, 32, 22, 17, 16 hours, respectively, whereas *Heteroclarias* exposed to 20, 30, 40, 45 and 50 ppm of Ariel detergent showed mean overturning times of 92, 50, 27, 24 and 20 hours, respectively (Tables 1 and 2).

Different responses were observed in the two toxicants which included uncoordinated swimming, swimming with head up and tail down, swimming upside down, secretion of mucus as compared to the control fishes which showed no loss of equilibrium or righting balance.

Survival time. Survival times were also observed to decrease with increase in concentration. Test organisms exposed to 20, 30, 40, 45 and 50 ppm of Omo detergent showed mean survival times of 92, 35, 23, 18 and 17 hours, while those exposed to 20, 30, 40, 45 and 50 ppm concentrations of Ariel detergent showed mean survival times of 95, 53, 38, 29 and 23 hours, respectively (Tables 1 and 2).

Responses of *Heteroclarias* fingerlings at these times included slow breathing rate, and quiescent behviour.

Regression analyses performed on transformed mortality data showed that % mortality (probit) depended strongly on concentration. A coefficient of determination, r^2, of 0.9925 was obtained for Omo detergent while a coefficient of determination, r^2, of 0.9882 was obtained for Ariel detergent; both indicating strong linear relationships

between concentration and mortality. The slopes of each regression line were found to be highly significant using students' t-test ($p < 0.05$).

Table 1

Time-effect relationships of fingerlings of *Heteroclarias* exposed to concentrations of Omo detergent

Concentration (ppm)	*Mean manifestation time (Hrs)*	*Mean overturning time (Hrs)*	*Mean survival time (Hrs)*
0.00 (control)	-	-	96.00
20	62.00	80.00	92.00
30	28.00	32.00	35.00
40	16.00	22.00	23.00
45	14.30	17.00	18.00
50	14.00	16.00	17.00

Table 2

Time-effect relationship of fingerlings of *Heteroclarias* exposed to concentrations of Ariel detergent

Concentration (ppm)	*Mean manifestation time (Hrs)*	*Mean overturning time (Hrs)*	*Mean survival time (Hrs)*
0.00 (control)	-	-	96.00
20	70.00	92.00	95.00
30	46.00	50.00	53.00
40	35.00	27.00	38.00
45	19.00	24.00	29.00
50	14.00	20.00	23.00

LC_{50}. The mean LC_{50} at 95% confidence limits of *Heteroclarias* exposed to Omo detergent was determined as 34.11 ± 1.08 with an LC_{50} interval of 33.03-35.19 ppm while the mean LC_{50} at 95% confidence limit of *Heteroclarias* exposed to Ariel detergent was determined as 38.66 ± 1.13 with an LC_{50} interval of 37.43-39.79 ppm (Table 3). A statistical comparison of mean LC_{50s} showed no significant differences ($p > 0.05$).

Table 3

Mean LC_{50} with 95% confidence limits of *Heteroclarias* fingerlings exposed to concentrations of Omo & Ariel detergents

Detergents	*LC_{50} at 95% confidence limits (ppm)*		
	Mean LC_{50}	*Lower limit*	*Upper limit*
Omo	34.11 ± 1.08	33.03	35.19
Ariel	38.66 ± 1.13	37.43	39.79

No adverse behavioural changes or any mortalities were recorded in the control experiments throughout the period of the static bioassay. Normal behaviour and colour was observed in the fishes all through the toxicity test. However, in the Omo and Ariel treated fishes, several abnormal behavioural responses were observed and recorded such as incessant jumping and gulping of air, restlessness, frequent surface to bottom movement, sudden change of direction during movement, resting at the bottom, loss of skin coloration, loss of equilibrium and gradual onset of inactivity.

Discussion. The results show that Omo with a lower mean LC_{50} of 34 ± 1.08 ppm is more toxic than Ariel with a mean LC_{50} of 38.66 ± 1.13 ppm. The t-test analysis showed significant differences ($p < 0.05$) in manifestation time of Omo and Ariel detergents at 50 ppm concentrations. But there were no significant differences ($p > 0.05$) recorded in other concentrations. There were no significant differences ($p > 0.05$) in overturning and survival times of the fish in the Omo and Ariel detergents in all the concentrations. Detergents, including the biodegradable ones have been discovered to induce poisonous effects and osmo-regulatory imbalances in aquatic organisms especially if present in concentrations that exceed metabolic demand. Substances such as xenobiotic compounds

in these detergents could be persistent and more mobile in soil and water; hence, they are known to be some of the most common terrestrial and aquatic contaminants (Cox 1998). Detergent effluents have also been noticed to induce severe damage to such vital organs like gills, kidneys, liver, skin, heart and the brain of animals (Omoregie et al 1990).

Warren (1971), Adewoye & Fawole (2002) and Adewoye et al (2005) had earlier reported that indiscriminate deposition of effluents into an aquatic systems might decrease the dissolved oxygen concentration which stands to impair respiration leading to asphyxiation and may ultimately result into organ architectural degradation such as liver dysfunction.

Observations from the present study show that during manifestation times the fishes showed abnormal behaviours such as hyperactivity. These behavioural abnormalities have been attributed to nervous impairment as a result of blockage of nervous transmission between the sensory receptors and various effector sites, enzyme disfunctions that may induce paralysis of respiratory muscles and/or depression of respiratory centre and disturbances in energy or metabolic pathways which result in depletion of energy (Avoajah & Oti 1997; Aguigwo 2002).

The small changes in the behavior of fish observed in the lower concentrations of Omo and Ariel detergent may be due to the avoidance behaviour of the test organism to the test substance. This conformed with the submission of Donaldson & Dye (1975) who were of the opinion that fish exposed to low concentrations of toxicants do not reach the threshold stage of exhaustion, rather, they quickly become adapted to the stressor.

The stressful and erratic behaviour of the fishes in this investigation may signal respiratory impairment and this may be as a result of the effects of the detergent on the gills. This is consistent with the opinion of Ogundiran et al (2009). At increased concentrations of Omo and Ariel detergent (0.45 and 0.5 pmm), the behavioural responses of the test organisms greatly increased and the organisms later became inactive, this is a normal situation in acute toxicity tests (Kulakkattolickal & Krammer 1997). Hyperactivities observed in this study are attributed probably to the disturbances in the metabolic state resulting in the depletion of energy (Ogundiran et al 2009).
Lethargies and loss of equilibrium observed in this study may be due to depletion of energy in the body of the exposed fishes. Also, lethargies and loss of equilibrium as recorded in this work could be an indication of impairment of normal carbohydrate metabolism and is a possible result of hormonal impairment (Anderson et al 1988).

It was also observed that the rate of mortality became greatly increased with increase in the concentration of the detergents. This is a reflection of what Fryer & Makepeace (1977) reported regarding all categories of toxicants; a threshold is reached at which there is no survival of the animal. An animal lies within a tolerable zone only below the threshold, but above the tolerance zone is the zone of resistance.

Detergents which end up in the aquatic environment through indiscriminate use, careless handling, accidental spillage or discharge of untreated effluents into natural water-ways have harmful effects on the fish population and other forms of aquatic life and ultimately humans who make use of the water for various purposes such as drinking, cooking, bathing, washing, etc. The results from this study with respect to manifestation times, overturning times and survival times show that the studied detergents are toxic to fish and that Omo is more toxic than Ariel. Even at the lowest concentration, it could have a significant toxic effect on fishes and other aquatic lives. For these detergents to have significant toxic effects even at low concentrations, it means that utmost care must be taken in using them in order not to exceed the Federal Environmental Protection Agency (FEPA) (1991) specification and safe limits for effluents discharged into all categories of water bodies.

Conclusions. This study has been able to establish the fact that Omo detergent (Unilever Nigeria Plc) is more toxic than Ariel detergent (Procter & Gamble Nigeria Limited) and that exposure of the *Heterobranchus longifilis* x *Clarias gariepinus* hybrid to even low concentrations of Omo and Ariel detergents can induce various toxicological

effects. In view of the toxic effects of these detergents, it can be inferred that indiscriminate discharge can be detrimental to aquatic fauna.

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