

# Lethal toxicity of industrial chemicals to early life stages of *Tilapia guineensis*

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## Abstract

The toxic effects of industrial chemicals on three early life stages of an economically important fish, *Tilapia guineensis* were investigated using the Organisation for Economic Cooperation and Development (OECD) # 203 recommended semi-static renewal bioassay. The assessment was necessary for the uncontrollable disposal of Neatex (liquid detergent) and Norust CR 486 (corrosion inhibitor) into the Niger Delta environment of Nigeria. The estimated 96-h LC<sub>50</sub> for 7-, 14- and 28-day-old fish in Norust CR 486 exposure was considered “more toxic” than Neatex in all life stages and was dependent on species age, exposure duration and environment. In the fresh water test, for Neatex and Norust CR 486 exposures for day 7, 14 and 28, the 96-h LC<sub>50</sub> were 8.79, 17.10 and 82.42 mg/l and 5.55, 13.58 and 20.21 mg/l, respectively. In the brackish test, 15.42 and 46.52 mg/l, not determined (ND) and 7.35, 13.95 and 24.50 mg/l were obtained. Differential toxicity was observed in the fresh and brackish water fish for the two chemicals and controls at  $p < 0.05$ . The high sensitivity of the 7-day-old test organisms to both chemicals provides a rationale for regulatory surveillance and monitoring of both chemicals in the fragile Niger Delta environment.

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## 1. Introduction

Our environment is currently flooded with chemicals that contaminate our air, water, food and humans. Discharge of these toxic chemicals into the environment cause adverse effects on biological systems, ranging from cell to ecosystem in varying ranges of direct and/or indirect effects. The degree of the effect depends on the type, property dosage and exposure duration of the chemical. Aquatic systems reflect perturbations in the environment, hence fish and invertebrates can often be used to indicate the health of an aquatic system because chemicals can be accumulated in fish and cause harmful effects [1].

Detergents have been used extensively as surface active agents in industrial and domestic premises to wash equipment, installations, heavy-duty machines, vehicles and oil soiled mate-

rials. They are also used in pesticide formulations and for dispersing oil spills at sea [2]. Corrosion inhibitors are used in a wide range of applications, such as oil pipelines, domestic central heating systems, industrial water-cooling systems and metal extraction plants.

Detergents find their way into surface water via sewage works and as detergent concentrations approach 15 parts per million, fish kills occur [3,4]. All detergents destroy the external mucus layers that protect the fish from bacteria and parasites causing severe damage to the gills. Their toxic effects on aquatic resources such as fish have necessitated the need for regulatory monitoring of water bodies that receive effluent containing these chemicals.

A major component of detergent and corrosion inhibitor is linear alkylbenzene sulphonate (LAS). LAS also called surface-active agents or wetting agents are organic chemicals that reduce surface tension in water and other liquids. Reports have shown that LAS is usually poorly broken down in rivers and may be toxic to aquatic organisms [5]. Hazardous effects of cationic, anionic and non-ionic detergents on aquatic organisms have been reported [6–8]. Previous investigation on toxicity of industrial

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detergent and corrosion inhibitors also include the *studies* of Whitehouse et al. [9,10].

Ecological risk assessments are usually conducted for the purpose of defining the extent of hazardous waste contamination in the aquatic biota. In the Niger Delta zone of Nigeria, existing data on environmental impact assessment reports by some oil companies are lacking in ecological risk assessment of industrial chemicals including the chemicals used in the present study despite their ecological impact and frequent use in the petroleum industries [11,12]. Bioassays are used to measure the degree of existing biological damage to fish, benthic organisms and other organisms using mortality, impaired physiology, biochemical abnormality and behavioural aberration as assessment end point [13]. In this regard, lethality test are particularly useful in the predictive assessment of environmental quality of chemicals discharged so that substantial safety factors and margins can be met.

The objective of this study was to assess the toxicity of two commonly used industrial detergent (Neatex) and corrosion inhibitors (Norust CR 486) to *Tilapia guineensis*. The suitability of fish (*T. guineensis*) in this study was based on the recommendation of Beeby [14]. They were chosen because they are highly prolific and available all year round. Other choices include; ease of maintenance under laboratory condition, relative sensitivity to toxic substances *furthermore* they are consumed by humans.

The monitoring of these effects is extremely important to regulate and remediate pollution. This is with the view of proposing a regular environmental monitoring programme for these chemicals in the Niger Delta *coastal* region of Nigeria, which has the highest number of petroleum activities and pollution problems.

## 2. Materials and methods

### 2.1. Collection of test organisms

*T. guineensis* (fish) from fresh and brackish environment of the Nigerian Niger Delta ecological zone were collected from a cultured fresh and brackish water farms at Kpakama and Abua in the Niger Delta area, respectively. The test organisms (7-day old) were collected on the first day of hatching while the other life stages (14- and 28-day) were collected 7 days before starting the test. The organisms were acclimated to laboratory conditions for 7 days before starting the test in holding tanks with dimensions length  $\times$  height  $\times$  width = 100 cm  $\times$  100 cm  $\times$  100 cm. The semi-static with renewal bioassays were conducted in amber coloured wide-mouth glass tanks measuring 40 cm  $\times$  25 cm  $\times$  25 cm.

The physico-chemical condition of the test water includes: temperature at  $26 \pm 2$  °C with a 16:8 h light:darkness photoperiod. The pH was  $5.5 \pm 0.2$  for the fresh water experiment and  $7.2 \pm 0.8$  pH units for the brackish water test and dissolved oxygen had a range of  $6 \pm 0.3$  mg/l. Salinity in the fresh test was  $59.72 \pm 3.4$  mg/l while the brackish water experiment had concentrations of  $3758 \pm 207$  mg/l. *Total dissolved solids (TDS)* and conductivity in the fresh water test was  $86.81 \pm 2.5$  mg/l

Table 1

The physico-chemical characteristics of the chemicals as contained in the materials and safety data sheet (MSDS)

Properties	Neatex	Norust CR 486
State or form	Liquid	Liquid
Colour	Light brown	Colourless
Odour	Pleasant	Pungent
Composition	Linear alkyl benzene sulphonate, sodium hydroxide, sodium carbonate and ammonium oxalate	Heterocyclic derivatives, linear alkyl benzene sulphonate and alkaline sulphide in ethylene glycol
Solubility	Soluble	Soluble
Specific gravity	1.04	1.09

and  $176.23 \pm 6.8$   $\mu$ S/cm, respectively. The brackish water experiment recorded  $6903 \pm 46.6$  mg/l and  $13814 \pm 88.61$   $\mu$ S/cm, respectively. These physico-chemical conditions are similar to that of the Niger Delta waters [11,12].

### 2.2. Test chemicals

The chemicals were collected from the manufacturers (Manuex Nigeria Limited and Ceca Incorporated) with the trade names Neatex and Norust CR 486, respectively. Both chemicals are currently used by oil industry operators in the Nigeria Niger Delta area. The constituents for the two industrial chemicals used for the 96-h acute toxicity test are given in Table 1.

### 2.3. Bioassay procedure

The semi-static renewal bioassay procedure started with a range finding test [15–17]. This was used to determine the range of concentrations to be tested and approximate the range that would produce the desired effective concentration EC<sub>50</sub> for the different life stages. The screening test was carried out with five different concentrations of the test chemicals.

Stock solutions of 200 mg/l were prepared by dissolving the chemicals in the dilution water *from* which serial dilution of 6.25, 12.5, 25, 50 and 100 mg/l were made. A total of 5 l of the test medium and controls (dilution water) was used to test 10 test organisms of *T. guineensis* for both fresh and brackish environment in three replicates. The fish were not fed 24 h before test initiation and during the 96 h of the test while aeration was for the test duration [18]. The test solutions were renewed daily and their physico-chemical constituents measured throughout the duration of the experiment.

### 2.4. Mortality

During the 96 h exposure period, mortality was recorded at 24, 48, 72 and 96 h. The dead organisms were removed immediately on detection. Fish were considered dead when they fail to show evidence of opercular activity and do not respond to gentle prodding [15].

Table 2  
Acute toxicity profile of fresh and brackish water fish to Neatex exposure

Days	LC <sub>50</sub> (mg/l)	Confidence limit	Probit equations	Slope
Fresh water				
7	8.79 ± 0.62	2.41–14.80	$Y = 3.22 + 1.82 \log x$	3.35 ± 0.27
14	17.10 ± 3.31	2.91–42.67	$Y = 3.59 + 1.13 \log x$	7.33 ± 1.09
28	82.42 ± 1.66	47.56–93.16	$Y = 1.40 + 1.88 \log x$	3.39 ± 0.32
Brackish water				
7	15.42 ± 0.92	2.94–32.96	$Y = 3.52 + 1.25 \log x$	6.55 ± 1.76
14	46.52 ± 2.16	26.88–129.66	$Y = 2.25 + 1.65 \log x$	3.99 ± 0.08
28	ND	ND	ND	ND

where  $Y$  = probit,  $x$  = concentration in mg/l, ND = not determined due to insufficient mortality to carry out the probit analysis.

### 2.5. Statistical analysis

The susceptibility of fish to both chemicals was determined using the probit method of analysis for median LC<sub>50</sub> at 96 h [19]. Computations of confidence interval of mortality rate were also obtained from the probit analyses.

## 3. Results

The results of acute toxicity of Neatex and Norust CR 486 to 7, 14 and 28-day-old *T. guineensis* are discussed below.

### 3.1. Mean percentage mortality

Mean % mortality was observed to be concentrations dependent. The influence of exposure duration and environmental conditions were also observed. Mortality increased as concentrations increased and it was higher in the fresh water experiment in both chemicals. In all control experiments no mortality was recorded. Mean % mortality at 96 h exposure in the freshwater and brackish water fish was significantly different at  $p < 0.05$  for both chemicals. The percentage mortality in control experiments was significantly different at  $p < 0.05$  for both chemicals.

### 3.2. Estimated 96-h LC<sub>50</sub>

Acute toxicity of both chemicals was evaluated using estimated 96 h LC<sub>50</sub> values in varying concentrations. Estimated 96 h LC<sub>50</sub> values for Neatex and Norust CR 486 varied in fresh and brackish water test (Tables 2 and 3). In Neatex estimated

Table 3  
Acute toxicity profile of fresh and brackish water fish to Norust CR 486 exposure

Days	LC <sub>50</sub> (mg/l)	Confidence limit	Probit equations	Slope
Fresh water				
7	5.55 ± 0	0.39–10.19	$Y = 3.59 + 1.90 \log x$	3.32 ± 0
14	13.58 ± 1.15	5.77–22.53	$Y = 2.89 + 1.86 \log x$	3.55 ± 0.79
28	20.21 ± 2.98	10.39–34.98	$Y = 2.68 + 1.78 \log x$	3.14 ± 0.33
Brackish water				
7	7.35 ± 0.27	1.70–12.28	$Y = 3.21 + 2.07 \log x$	3.03 ± 0.18
14	13.95 ± 0.80	6.68–22.38	$Y = 2.57 + 2.11 \log x$	3.14 ± 0.33
28	24.50 ± 2.82	14.47–41.32	$Y = 2.19 + 2.03 \log x$	3.10 ± 0.25

96 h LC<sub>50</sub> ranged between 8.79 mg/l (freshwater) and 15.42 mg/l (brackish water) while in Norust CR 486, 96 h LC<sub>50</sub> for the day 7 fish, ranged between 5.55 mg/l (freshwater) and 7.35 mg/l (brackish water). In the Neatex exposure for day 14 *T. guineensis*, the estimated LC<sub>50</sub> in the fresh and brackish water test was 17.10 and 46.52 mg/l, respectively while in the Norust CR 486 experiment, the LC<sub>50</sub> varied between 13.58 and 13.95 mg/l for both environments. Estimated LC<sub>50</sub> obtained for the 28-day-old fish exposed to Neatex concentration in the fresh water test was 82.42 mg/l while the LC<sub>50</sub> could not be determined in the brackish test due to insufficient mortality observed. In the Norust CR 486 experiment for fresh and brackish water, the estimated LC<sub>50</sub> for the 28-day-old *T. guineensis* varied between 20.21 and 24.50 mg/l, respectively. The estimated 96 h LC<sub>50</sub> values showed that Norust was more toxic than Neatex in both fresh and brackish water fish. The freshwater fish were more sensitive to both chemicals. Probit analysis also showed that LC<sub>50</sub> values decreases with increase in concentrations of chemicals, indicative of an increase in toxicity with increase concentration.

## 4. Discussion

In this study fish fingerlings of *T. guineensis* an economically important cichlid were exposed to two commonly used chemicals (industrial detergents and corrosion inhibitors), which are normally discharged into the fresh and brackish water environment in the Niger Delta region of Nigeria where industrial activities are intense. *T. guineensis* occurs in the fresh and brackish waters of the Niger Delta ecozone and are constantly exposed to these contaminants.

The effective concentrations that were observed for the surfactant-containing test chemicals in the present study varied with the life stages. Numerous studies have been performed to determine the effects of anionic surfactants and surfactant-containing chemicals towards aquatic organisms and these include the studies of Madsen et al.; Scarlett et al. and Belanger et al. [6,7,20]. Understanding the toxic mechanism of a contaminant helps evaluate the importance of potential exposure pathways and selection of sensitive ecological receptors. For instance, a contaminant (like surfactant and surfactant-containing chemicals) may selectively affect lower and higher vertebrates as well as invertebrates by interfering with the respiratory systems, or be present at a level that may be toxic to most organisms and threaten top predators through food chain. Surfactants in surface water have become an environmental concern and, studies on their effects on freshwater and marine life started since the early 1950s [21]. However, the varying degree of mortality reported in this study is supported by Bury et al. [22] who reported that differences in an organism's biological adjustment and behavioural response to changes in water chemistry and osmotic conditions depend on the stage of development. The implication of this observation is that the early life stages are not only vulnerable to chemical contaminants but are usually adversely affected. Survivorship depends on the degree of prolificacy of the parent organisms.

Differential acute toxicity levels observed for both chemicals are a reflection of varying degree of sensitivity of test organ-

isms to exposure concentration and duration. Test organisms showed better tolerance to lower concentrations of chemicals, which does not necessarily mean complete compensation for the chemical [23]. In order to assess the potential hazard of these chemicals to *T. guineensis*, toxicity data should be related to expected or actual environmental exposure concentrations. Because of lack of information on measured concentrations of these chemicals in aquatic systems, toxicity endpoints data were compared to the GESAMP rating [24]. The estimated 96 h LC<sub>50</sub> values obtained in this study showed that both chemicals are slightly toxic to the early life stages of fish.

Neatex and Norust CR 486 exposure in the fresh water test was ‘more toxic’ than the brackish water test. This result is consistent with other related studies [20,25,26]. The relative difference observed in the mean % mortality and 96 h LC<sub>50</sub> values between the fresh and brackish water test may not be unconnected with the varying osmoregulatory demand of the different environment. It has been reported that in the fresh water environment, any physical damage to external tissues allows more water to enter the body (and salt to escape), placing an additional burden on the kidneys, ultimately resulting in death [22]. This observation probably accounts for the higher mortality in the fresh water test. It has also been reported that toxicity of chemicals can be altered by variations in water chemistry by affecting their amount of the chemical available to bind to the fish [22,27,28].

Multiple stressors of varying sources and intensity are bound to affect organisms living and breeding in aquatic environment as a result of constant exposure due to uncontrollable discharges of industrial chemicals into such water bodies. Mortality of *T. guineensis* exposed to varying concentrations of Neatex and Norust CR 486 were influenced by toxicity modifying factors such as exposure duration, concentrations, type of chemicals, life stage and environmental conditions. In all concentrations, organisms showed varying degrees of stress to Neatex and Norust exposure. This study reported the vulnerability of early life stages of *T. guineensis* fingerlings to chemical contaminants.

## 5. Conclusion

The public concerns over the safety of industrial chemicals to the user and environment is at all-time high. The lethal concentrations of these surfactant-containing test chemicals obtained in this study are based on observations over a 4-day period in the laboratory. The significant difference observed in the mortality between the controls and the test concentrations suggests that the chemicals may be the cause of mortality in of the fish. *These findings also suggest that* these chemicals should be discharge into the environment only after treatment. The results of this study justify the need for regulatory monitoring of chemicals discharged into the waters of the Niger Delta ecological zone. The slightly toxic nature of acute exposure of *T. guineensis* to Neatex and Norust CR 486 may infer risk. However, results of the acute exposure to higher concentrations shows that the chemicals may have the potential to impact fish populations and warrants stiff regulatory compliance.

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## Glossary

*Acclimation:* To accustom experimental organisms to the biological, chemical and physical conditions present during holding, culture and testing. The term usually refers to controlled laboratory conditions.

*Acute toxicity:* A discernible adverse effect induced in the test organisms within a short period of exposure to a test material, usually  $\leq 4$  days for fish.

*Control:* A treatment in an investigation or study that duplicates all the conditions and factors that might affect the results of the investigation, except the specific condition that is being studied. In an aquatic toxicity test, the control must duplicate all the conditions of the exposure treatment (s), but must contain no test material.

*Control/dilution water:* The water used to dilute a test material in order to prepare different concentrations for the various toxicity test treatments.

*Culture:* The animals which are raised on-site or maintained under controlled conditions to produce test organisms through reproduction.

*EC50:* The median effective concentration estimated to affect 50% of a test population during continuous exposure over a specified period of time.

*Endpoint:* The variables (i.e., time, reaction of the organisms, etc.) that indicate the termination of a test, and also means the measurement (s) or value (s) derived, that characterize the results of the test (LC50, etc.).

*LC50:* The median lethal concentration (i.e., the concentration of test substance that is estimated to kill 50% of a test population during continuous exposure over a specified period of time). The LC50 and its 95% confidence limits are usually derived by statistical analysis of mortalities in several test concentrations, after a fixed period of exposure. The duration of exposure must be specified (e.g., 96 h LC50).

*Lethal:* Causing death by direct action. Death of fish is defined as the cessation of all visible signs of movement or other activity.

*Mortality:* The death of experimental organisms as a result of exposure to toxic substances present in soil, sediment or water. Usually expressed as percentage.

*Photoperiod:* The duration of illumination and darkness within a 24-h day.

*Semi-static renewal:* Toxicity tests in which test solutions are renewed during the test, usually after 24 h.

*Stock solution:* A concentrated aqueous solution that can be stored. Measured volumes of a stock solution are added to dilution water in order to prepare the required strengths of solutions.

*Toxicity:* The inherent potential or capacity of a material to cause adverse effects on a living organism.

*Toxicity test:* A determination of the effect of a material on a group of selected organisms under defined conditions. An aquatic toxicity test usually measures the proportions of organisms affected by their exposure to specific concentrations of chemical, effluent, elutriate, leachate, or receiving water.

*Toxicology:* The study of the harmful effects of substances on humans or animals.