African Journal of Biotechnology Vol. 9 (12), pp. 1839-1843, 22 March, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB08.991
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Sublethal effects of industrial chemicals on fish fingerlings (*Tilapia guineensis*)

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Accepted 30 June, 2009

Tilapia guineensis **commonly found in the Niger Delta ecological zone of Nigeria was exposed to sublethal concentrations (1.56, 3.13 mg/l) of neatex (industrial detergent) and norust CR 486 (*corrosion* inhibitor) using the Organisation for Economic Cooperation and Development (OECD) # 203 protocol. At test termination on the 28th day, the rate of bioaccumulation of surfactants in the fish gills, gut and muscle tissues were measured. The levels of surfactant in the gills, gut and muscle tissues were significantly different at levels of $p < 0.05$ in fish exposed to neatex and norust CR 486. Surfactant levels in the fish also increased significantly with increase in concentrations. Surfactant accumulation in the test treatments may be an indication that the observed effects on the exposed fish may have been due to the chemicals. This study demonstrates the sublethal effects of surfactant-containing industrial chemicals on *T. guineensis*, an economic and ecologically important sentinel.**

Key words: *Tilapia guineensis*, industrial chemical, bioaccumulation, surfactants.

INTRODUCTION

In everyday life one frequently comes into contact with surfactants (surface active agents). Surfactants (anionics, nonionics, cationics and amphoterics) are used in various premises for a number of different purposes. In food products, naturally occurring surfactants act as emulsifiers, for example casein in milk and other dairy products. In our households, synthetic surfactants are active ingredients in detergents, cosmetics and pharmaceuticals. In industry, surfactants are used in pulp and paper manufacturing, in oil recovery, in flotation where different minerals are separated from each other and a host of other applications (Rapaport, 1995).

Surfactants are amphiphilic molecules consisting of 2 different parts: one hydrophilic (“water loving”) and the other hydrophobic, (“water rejecting”). This combination makes the surfactant ambivalent; the hydrophilic head group is attracted to polar environments, for example water, while the hydrophobic tail is attracted to nonpolar environments, for example oil (Ghazali and Ahmad, 2004). Linear alkylbenzene sulphonates (LAS) are the most widely used group of anionic surfactants. Neatex (industrial detergent) and norust CR 486 (corrosion inhibitor) are formulated products, containing LAS, which facilitate dirt, stain and soil removal from surfaces or equipments and are used to achieve a clear liquid product that has acceptable stability in oil pipelines (Patton, 1995).

Most organic chemicals are lipophilic and thus have the tendency to bioaccumulate. Bioaccumulation is the "building-up" of a chemical to a toxic level in an organism's body. Bioaccumulation becomes an environmental problem when chemicals accumulated are toxic and lead to an elevated amount in the organism’s body (Heng et al., 2004). Linear alkylbenzene sulphonate (LAS), have hydrophobic components, which may partition into lipids tissues of organisms and bioaccumulate. If the surfactetants are not catabolized, the possibility exists for magnification of potential toxicological effects up the food chain (Britton, 1998).

All the major surfactant groups (anionics, nonionics, cationics and amphoterics) are currently used to a large extent in industrial and domestic premises. However, only minimal experimental and monitoring information has

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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

been gathered on the bioaccumulation properties of some of the currently used surfactants (McWilliams and Payne, 2001). Residual amounts of surfactants typically found in many industrial and household cleaning formulations continue to find their way into our waterways. They are typically discharged into the environment through the sewage treatment infrastructure (that is, sewers, sewage treatment plants), or directly in situations where no treatment systems are available (Akyuz and Roberts, 2002). Their use and disposal often contaminate the aquatic ecosystem, which usually acts as a recaptacle of effluent.

Sublethal concentrations of surfactants at 0.005 mg/l have demonstrated adverse effects on aquatic species (Misra et al., 1991). LAS could potentially harm organisms by denaturing proteins and depolarising cell membrane (Pozo et al., 2003). Abel (2006) reported that sublethal effects of surfactants include retardation of growth, alteration of feeding behaviour and inhibition of chemoreceptor organs. Low levels of detergents may also increase the uptake of other pollutants. The interactions between detergents and proteins and their influence on membrane permeability may be the basis of the biological action of detergents. Studies of sublethal levels of LAS in fish show that the gills and the locomotive muscles are the most susceptible sites of surfactant toxicity. Tadros (1984) reported that early developmental stages of feeding sac-fry are very susceptible to LAS.

Linear alkylbenzene sulphonate (LAS) is rapidly aerobically degraded, but only very slowly and not at all degraded under anaerobic conditions (Sanchez-Peinado et al., 2008, 2009; Schowanek et al., 2007). Although LAS are readily degradable in aerobic conditions, a consortium of microorganisms is needed to effectively carry out the degradation. In this vein gram negative bacteria were able to tolerate LAS even though they did not degrade them because of the protective phospholipid coating (Sanchez-Peinado et al., 2008, 2009). Therefore, LAS can be found in very high concentrations in most sewage sludge but a half-life of approximately 1-3 weeks will generally prevent accumulation in soil and biota, if exposure is not continuous (Prats et al., 2006).

Since fish are known to be affected by exposure to industrial chemicals in the ambient environment to levels that could be deleterious to the organisms, this study aimed to demonstrate the effects of sublethal concentrations of neatex and norust CR 486 on the gills, gut and muscle tissues of *Tilapia guineensis*, which are of economic and ecological significance in the Niger Delta of Nigeria (DPR, 2002).

MATERIALS AND METHODS

Collection of test organisms

T. guineensis (fish) were collected from a fresh water farm at Kpakiama in the Niger Delta ecological zone of Nigeria. The characteristics of the organisms include the following: Age, 28 day old; weight 1.19 ± 0.04 g and size 3.82 ± 0.15 cm. The organisms were acclimated to laboratory conditions for 7 days prior to the test in holding tanks measuring 100 x 100 x 100 cm.

Test chemical

Two test chemicals namely neatex and norust CR 486 were purchased from the manufacturers, Manuex Nigeria limited and Ceca incorporated, Lagos, Nigeria respectively. The test chemicals currently used by oil industry operators in the Nigeria Niger Delta area are soluble liquids with linear alkylbenzene sulphonates (LAS) as a major active constituent, approximately 12 - 16% in neatex and 25 - 27% in norust CR 486 (Table 1).

Bioassay procedure

The Organisation for Economic Cooperation and Development (OECD) # 203 (1992) protocol was employed for the semi-static renewal bioassay. The sublethal bioassay was carried out in amber coloured glass tanks measuring 40 x 25 x 25 cm. This was estimated using the surfactant components (LAS) in the test chemicals to assess the long term effect on 28 day old *T. guineensis* with 2 concentrations (1.56 and 3.13 mg/l) in triplicate. The control experiment contained the dilution water (that is, water from the organisms' habitat) and the fish samples. Each aquarium contained 10 fish samples. The surfactant levels in the fish were estimated at test termination (28 days).

The test solutions were renewed daily and their physico-chemical constituents were measured throughout the duration of the experiment. The mean pH and salinity of the test solutions determined at test initiation (day 0) and test termination (day 28) were 5.9 ± 0.2 pH units and 0.06 ± 0.003 ppt respectively. Total dissolved solids (TDS) and conductivity were 86.81 ± 2.5 mg/l and 176.23 ± 6.8 µS/cm, respectively.

Table 2. Bioconcentration factor of surfactant in neatex and norust CR 486 exposure at 28 days.

Concentration	Bioconcentration factor	
	Mean Neatex exposure ± SD	Mean Norust CR, 486 exposure ± SD
1.56 mg/l, Gill	98.14 ± 9.40	94.89 ± 7.30
1.56 mg/l, Gut	118.01 ± 12.10	106.93 ± 7.10
1.56 mg/l, Tissue	147.83 ± 2.20	137.23 ± 0.60
3.13 mg/l, Gill	125.43 ± 3.40	96.48 ± 0.90
3.13 mg/l, Gut	144.83 ± 3.40	138.94 ± 3.90
3.13 mg/l, Tissue	160.78 ± 4.50	253.42 ± 3.00

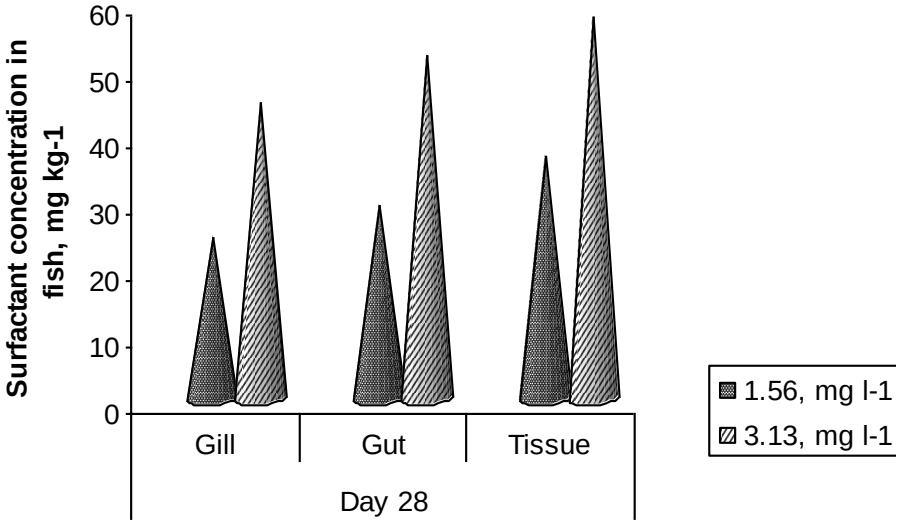

Figure 1. Surfactant bioaccumulation of neatex at 28 days.

Estimation of the surfactants (LAS) levels in fish

Extraction of surfactant (LAS) from fish organs and tissues

The gills, gut and muscle tissues of the fish were weighed (100 mg) and placed in extraction containers. The surfactant in the gills, gut and muscle tissues were extracted for over 16 h with 30 ml methanol. The extracts were evaporated to dryness in the extraction vessels and the dry residues were dissolved with 100 ml of warm deionised water in a water bath (Knepper et al., 1999). The redissolved solutions containing extract of the fish organs and muscle tissues were used to determine the surfactants levels following the procedure described in APHA (1995) and Zaporozhets et al. (1998).

Surfactant determination

The surfactant levels (LAS) in 100 ml of the fish extracts, test solutions and standards (0.25, 0.5, 1.0 and 2.0 mg/l) were determined using methylene blue method (APHA, 1995; Zaporozhets et al., 1998). The method comprised of 3 successive extractions of the solutions (extracts, test solutions and standards) to which 25 ml methylene blue reagent and 10 ml chloroform have been added. The chloroform layer was drawn off into a second separatory funnel and a further extraction was done using 50 ml of wash solution (50 g sodium phosphate and 41 ml 3 M sulphuric acid in 1000 ml). The surfactant content in the solutions was determined by measuring the absorbance of the samples and standards against a blank of chloroform at 652 nm. The intensity of the blue colour in the chloroform layer is proportional to the amount of LAS present. The concentrations of surfactant in the samples were then extrapolated from the calibration graph (APHA, 1995; Zaporozhets et al., 1998).

Statistical analysis

Mean significant differences within a group in the sublethal bioassays were assessed with the two-factor ANOVA (analysis of variance).

RESULTS AND DISCUSSION

Results obtained for surfactant bioaccumulation levels are presented in Table 2 and Figures 1 and 2. Surfactants residues were measured in the gills, guts and muscle tissues of the fish at day 0 and 28 for both chemicals at concentrations of 1.56 and 3.13 mg/l. The results obtained showed that the fish organs and muscle tissues analysed had higher surfactant residues at concentrations of 3.13 mg/l. The estimated bioconcen-

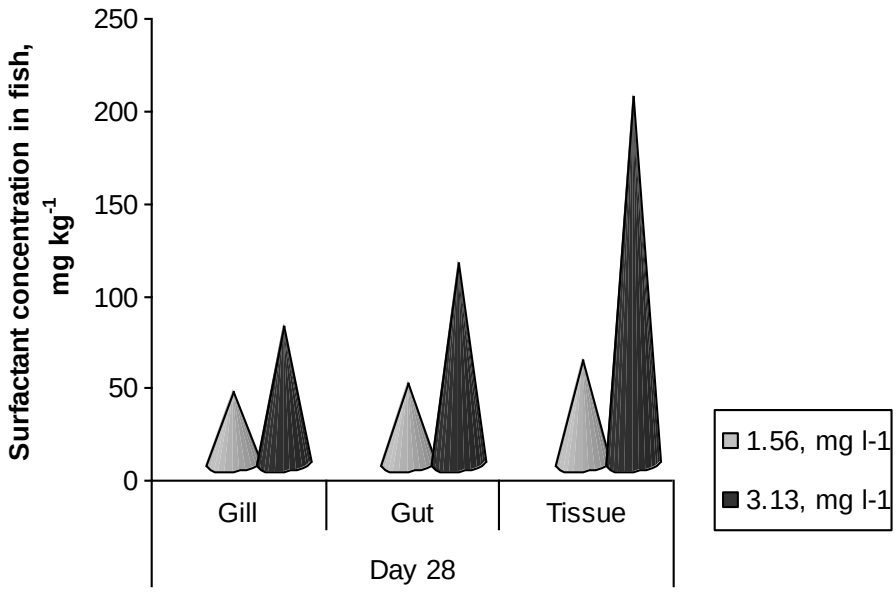

Figure 2. Surfactant bioaccumulation of norust CR 486 at 28 days.

tration factor (BCF) was higher in the norust CR 486 exposure when compared with neatex. The mean surfactant concentrations in the gills, gut and muscle tissues at the end of the 28 day experiment were significantly different at $P < 0.05$ for the both chemicals.

Surfactants generally seem to impact on higher aquatic organisms via their respiratory structures. In higher organisms such as fish the respiratory structures (gills) consist of epithelial membranes that may be extensively folded to provide large surface areas for gaseous exchanges. Destabilisation of these epithelial membranes, as may occur when exposed to surfactants, results in changes in membrane permeability, cellular lysis and impairment of cellular respiration (McWilliams and Payne, 2001). In lower organisms, in which exchange of respiratory gases is via mechanisms of simple diffusion across membrane surfaces, surfactant toxicity appears to result from an initial disruption of normal membrane function followed by physical disruption of the cellular membrane. Surfactants can be toxic to aquatic life at concentrations as low as 0.025 mg/l (Chattopadhyay and Konar, 1985). The concentrations observed in the organisms are an important approach to assess and evaluate their behaviour in the environment. Whitehouse et al. (1998) also observed similar results in their studies on surfactants.

The degree to which a contaminant will concentrate in an organism is expressed as a bioconcentration factor (BCF), which is defined as the concentration of a chemical in an organism's tissues divided by the exposure concentration. With regard to bioaccumulation, Whitehouse et al. (1998) found the bioconcentration factors for LAS in aquatic organisms to be around 300. Chemicals and substances with a BCF greater than 1000 tend to bioaccumulate and would be persistent in the medium of contamination (EPA, 2000). In this study bioconcentration potentials varied between neatex and norust CR 486. Although values obtained were less than 1000 in the 2 chemicals, sublethal effects, gives a true indication of the effects of chemicals on the tested organisms. Therefore, surfactants should always be scrutinized for potential bioaccumulation effects (Roberts, 1991; Dimitrov et al., 2002). Information on the dynamic process of bioaccumulation is very important in protecting humans and other organisms from the adverse effects of chemical exposure and it has become a critical consideration in the regulation of chemicals (DPR, 2002). Bioaccumulation is also recognized as an essential component of environmental toxicology risk assessment (SETAC, 1997).

Sanchez-Peinado et al. (2008 and 2009) have reported that LAS is degradable under aerobic condition, however, this does not eliminate its toxic effects to aquatic organisms if exposure is continuous. The time lag between accumulation and degradation should also be considered when LAS is released into the environment. If a reasonable concentration have been absorbed in the organism's organs before elimination and the rate of absorption is greater than elimination, then accumulation would occur (Abel, 2006). The obvious toxicological effect in this study was slight LAS accumulation in the organs and tissues of the organisms, which could possibly lead to other adverse effects due to intake through gills and adsorption in tissues. The effects of surfactant bioaccumulation on glycogen reserves have been reported by Ezemonye et al. (2007). The quantity of LAS in the environment as well as the duration of degradation (1 - 5 days) are useful in determining the effects since the organisms are continually exposed to the experimental concentrations. There is the likelihood that such orga-

nisms may not survive beyond a particular duration if exposure is continuous. In so much that there is the possibility of sudden death if they are deficient in muscle glycogen (Ezemonye et al., 2007). Glycogen reserves in the muscle tissues of fish under stressful conditions can be used as an emergency energy supply and any change observed in the tissues and organs, acts as an index of the organism's health status, especially in aquatic community (Cicik and Engin, 2005).

Conclusion

This study has shown that bioaccumulation of surfactants in the organisms could possibly affect their health status and impair vital processes if exposure is continuous and for a long period of time. Thus, the environmental safety assessment of surfactants and related products should focus primarily on the aquatic ecosystem since the ecological effects of these chemicals could be detrimental to organisms. Therefore, regular monitoring of the waters containing these chemicals frequently discharged into the Niger Delta area of Nigeria is required.

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